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Animal Models in Psychiatry, II

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Preface to the Series

When the President of Humana Press first suggested that a series on methods in the neurosciences might be useful, one of us (AAB) was quite skeptical; only after discussions with GBB and some searching both of memory and library shelves did it seem that perhaps the publisher was right. Although some excellent methods books have recently appeared, notably in neuroanatomy, it is a fact that there is a dearth in this particular field, a fact attested to by the alacrity and enthusiasm with which most of the contributors to this series accepted our invitations and suggested additional topics and areas. After a somewhat hesitant start, essentially in the neurochemistry section, the series has grown and will encompass neurochemistry, neuropsychiatry, neurology, neuropathology, neurogenetics, neuroethology, molecular neurobiology, animal models of nervous disease, and no doubt many more "neuros." Although we have tried to include adequate methodological detail and in many cases detailed protocols, we have also tried to include wherever possible a short introductory review of the methods and/or related substances, comparisons with other methods, and the relationship of the substances being analyzed to neurological and psychiatric disorders. Recognizing our own limitations, we have invited a guest editor to join with us on most volumes in order to ensure complete coverage of the field. These editors will add their specialized knowledge and competencies. We anticipate that this series will fill a gap; we can only hope that it will be filled appropriately and with the right amount of expertise with respect to each method, substance or group of substances, and area treated.

Alan A. Boulton
Glen B. Baker

Preface

The two *Animal Models in Psychiatry* volumes are loosely organized by subject. The first volume contains a number of chapters concerned with schizophrenia, psychoses, neuroleptic-induced tardive dyskinesias, and other disorders that may involve dopamine, such as attention deficit disorder and mania. Also included is a chapter describing a behavioral model for activity-induced anorexia. The second volume deals with affective and anxiety disorders, but also includes chapters on subjects not easily classified as either psychotic, affective, or anxiety-related, such as aggression, mental retardation, and memory disorders. Four chapters on animal models of schizophrenia or psychoses are included in Volume 18 because of the importance of these disorders in psychiatry. Likewise, three chapters in the present volume deal with affective disorders, with a fourth chapter on circadian rhythms that also contributes to methods for animal models in affective disorders.

Following the first four chapters are two chapters dealing with models of anxiety and panic, two chapters on aggression, one on mental retardation, and a final chapter covering memory disorders. Many of the behaviorally-based models of affective disorders involve inducing stress in animals, usually on a chronic basis. The first chapter by Anisman, Zalcman, Shanks, and Zacharko describes some of the neurochemical effects that are associated with the chronic application of sensors. Two important features of this chapter are the descriptions of stress–immune system interactions and the marked influence that an animal's ability to control external stressors has on both neurochemical and immune responses. Both of these phenomena have clear implications for the human condition. The next two chapters deal with models of major affective disorder that are based on surgical or chemical interventions, rather than on manipulations of environmental conditions. Richardson describes a model based on olfactory bulbectomies that appears to share many of the characteristics of depressive disorder, including many of the alterations in circadian rhythms seen in depressed patients. The chapter by Overstreet and Janowski describes an animal model of affective disorders that rests on new clinical evidence based on pharmacological challenges for an older theory of a cholinergic involvement in affective disorders. It may prove of interest for the investigation of a genetic contribution to depression, since it involves the breeding of mice with a supersensitive response to muscarinic agonists. Both of the above models of affective disorders have a pragmatic advantage over stress-based models in that they do not require submitting animals to procedures that may require more stringent ethical justifications. Interest in the involvement of circadian rhythms in psychiatric conditions has been increasing at a tremendous rate, largely as a result of the recent emergence of Seasonal Affective Disorder as a discrete diagnostic entity. The chapter on circadian rhythms is written by Yamada and Takahashi, two clinician-researchers with extensive experience in both animal and human circadian rhythm research. This survey of the available techniques for the analysis of biological rhythms will be extremely useful for those attempting to pursue research in this area. The next chapter presents a comprehensive review of a wide variety of animal models for the screening of anxiolytic drugs. Although it may appear to the reader that there is a plethora of reviews of this important area, Sanger includes descriptions of how the atypical anxiolytic, buspirone, has fared in these models. This nonbenzo-diazepine anxiolytic may prove to be instrumental in choosing models that screen for a general anxiolytic action, rather than for "benzodiazepine-like" actions. With the relatively recent separation of panic disorders from generalized anxiety disorders, the chapter by Commissaris and Fontana is timely. Human aggression is a problem of significance from both a psychiatric and a social perspective. The chapters by Sheard and by Koolhaas and Bohus describe techniques for assessing aggression in animals. Sheard's review of the methodology for drug studies in aggression is of interest not only in terms of screening for potential antiaggressive drugs, but for the determination of the neural substrates of aggression using locally infused or microiontophoretic application of drugs into specific brain regions. Koolhaas and Bohus have described the variety of types of aggression, with a comprehensive survey of the factors that can influence aggressive behaviors. The next chapter is a thorough review of animal models of mental retardation, which includes a description of a number of methods for inducing global CNS damage. Archer, Hard, and Hansen have provided an intriguing concept of a General Learning System that can be tested in animals with a variety of tasks in a modified shuttle box. This may provide part of a relatively simple test battery that maintains a comprehensive assessment of learning abilities. The last chapter deals with animal models of memory disorders. This topic has received an enormous amount of attention recently, with the cholinergic deficits in Alzheimer's disease attracting research into potential pharmacotherapies for memory deficits. The difficulty in attributing memory deficits to reductions in performance has been and remains extremely difficult. The excellent review by Overstreet and Russell describing methods of testing for memory deficits as well as techniques for inducing Alzheimer's disease-like neurochemical pathologies will aid in the selection of models in which the attribution of deficits to alterations in memory processes will be strengthened.

Mathew T. Martin-Iverson

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Multisystem Regulation of Performance Deficits Induced by Stressors

An Animal Model of Depression

Hymie Anisman, Steve Zalcman,
Nola Shanks, and Robert M. Zacharko

1. Introduction

When an organism is exposed to a stressor, a series of behavioral changes occur that are thought to be of adaptive value. Among other things, the response style of an organism will narrow to those innate responses highest in the animal's defensive repertoire (*see* Bolles, 1970) or to responses previously acquired in aversive situations. In addition, several neurochemical changes occur that may blunt the physical or psychological impact of the stressor, increase arousal or vigilance, or increase the animal's ability to initiate and sustain defensive responses (*see* reviews in Zacharko and Anisman, 1989; Maier and Seligman, 1976; Weiss and Simson, 1985). However, there may be occasions where these responses may have adverse consequences. For instance, when the response required to escape from the stressor is not part of the organism's repertoire, the persistent adoption of these response styles may be counterproductive. Likewise, excessive utilization may reduce neurotransmitter stores, rendering the animal less able to deal with environmental demands. It has been our contention that many of the behavioral and physiological

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disturbances associated with acute and chronic uncontrollable stressors stem from the failure of adaptive neurochemical mechanisms. This chapter will outline some of the biochemical and behavioral consequences of stressors, particularly as they relate to an animal model of depression.

The proposition that stressful events may influence the development or the course of clinical depression in humans is an intuitively appealing one. Both retrospective and prospective studies have, indeed, provided evidence commensurate with such a view, and have suggested that such factors as early life experiences, coping style, and personality variables may influence the impact of a stressor on affective state (e.g., Abramson et al., 1978). While supporting the contention that stressors may contribute to the provocation or exacerbation of clinical depression, we have expressed misgivings concerning some of the animal models that may be employed as analogs of the human clinical condition.

An animal model of any human disorder requires, among other things, that

1. The behavioral disturbances being assessed are reminiscent of the symptoms associated with the human pathology
2. The underlying mechanisms are comparable
3. The treatments effective in ameliorating the human disorder are also effective in animals and
4. Conversely, those treatments ineffective in eliminating the pathology in humans are also ineffective in eliminating the behavioral disturbances in animals.

In addition to the aforementioned considerations, an animal model of depression must ultimately come to account for the fact that:

1. Individual differences may exist in response to a stressor; not all individuals will perceive a stressor in the same way, and other things being equal, the response to a stressor may differ across individuals
2. The symptom profile associated with depression may vary appreciably across subjects and

3. Depression may be a biochemically heterogeneous disorder; pharmacological treatments effective in alleviating depressed symptoms in one individual may be ineffective in a second (*see* Anisman and Zacharko, 1989).

The aforementioned considerations have prompted investigators to reevaluate the approaches to developing animal models of depression. These include greater reliance on a series of different behaviors that, considered in conjunction with one another, more closely resemble the symptom profile of depression (e.g., Weiss and Simson, 1985; Willner, 1985), determination of the neurochemical concomitants of stressors and their relationship to behavioral disturbances (Zacharko and Anisman, 1989; Weiss et al., 1986), and analyses that focus on the contribution of genetic factors in vulnerability to stressor-provoked behavioral disturbances (Shanks and Anisman, 1989).

2. Neurochemical Consequences Associated with Environmental Insults

2.1. Norepinephrine

Exposure to a variety of different stressors will increase the utilization and synthesis of norepinephrine (NE) in several brain regions. If the stressor is a controllable one and of moderate severity, then the synthesis of the amine will keep pace with utilization, and concentrations of the transmitter will not be affected appreciably. If, however, behavioral methods of terminating the stressor are unavailable, then NE utilization will increase still further, thus resulting in a net decline of the amine. In effect, the animal's ability to control stressor termination appears to be fundamental in determining whether an NE reduction will be evident (Anisman et al., 1980; Weiss et al., 1976a). In addition, it appears that the predictability of a stressor influences NE activity. When shock was preceded by a signal, the accumulation of the NE metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG) was increased, but to a lesser extent than that produced by shock that was not systematically preceded by the signal (Tsuda et al., 1989). Evidently, the latter treatment was more effective in provoking increased NE utilization.

Using a Sidman avoidance procedure, Tsuda et al. (1983) and Tsuda and Tanaka (1985) observed that following either a 3- or 6-h test session hypothalamic MHPG levels were elevated, and NE concentrations reduced relative to nonshocked or inescapably (yoked) shocked rats. These effects were less pronounced in yoked rats than in the escapably shocked animals. In a subsequent experiment, in which 21 h of treatment were applied, MHPG was increased in the escapable group, but to a lesser extent than it was after 3 or 6 h of training, or in rats that received inescapable shock. These findings suggested that, when animals were exposed to a relatively severe stressor, NE utilization was increased appreciably, thus resulting in a decline of NE levels. However, once the task contingencies were mastered, the rate of amine utilization was curtailed and, consequently, NE concentrations approached control values.

Paralleling the finding that stressor controllability may influence NE turnover, there are several reports suggesting that under some conditions aggressive behaviors may serve as a coping style to prevent physiological changes ordinarily produced by stressors. For instance, Weiss et al. (1976b) reported that the ulcerative effects of footshock were minimized in rats that were permitted to fight, whereas Sklar and Anisman (1980) demonstrated that the tumor-enhancing effects of change in housing conditions were less pronounced in mice that engaged in fighting than in those mice that did not. Likewise, it was reported that the NE alterations ordinarily associated with footshock were limited in rats that were housed in pairs and engaged in shock-elicited fighting relative to rats that did not exhibit this behavior (Stolk et al., 1974). Moreover, the increased MHPG accumulation induced by restraint was minimized in rats given the opportunity to bite a wooden probe (Tsuda et al., 1988).

Not surprisingly, the effectiveness of a stressor in altering NE turnover and concentrations varies with the stressor severity and with the brain region examined. For instance, although immobilization readily provoked alterations of NE activity in hypothalamus, amygdala, and basal ganglia, a greater stressor severity was necessary to alter cortical and hippocampal NE turnover (Tanaka et al., 1982). Likewise, the stress of witnessing conspecifics receive footshock (as well as exposure to vocalization and odors) was found to alter NE turnover preferentially in the hypothalamus and amygdala of the rat (Iimori et al., 1982).

In assessing the relationship between central NE alterations and behavioral changes induced by stressors, Weiss and his associates (Simson et al., 1986a,b; Weiss and Simson, 1985; Weiss et al., 1981,1986) have devoted particular attention to the contribution of the locus ceruleus. These investigators have shown that NE reductions were induced readily in the locus ceruleus and, after a relatively severe stressor, may have persisted as long as 72 h (cf. 48 h in the hypothalamus). Inasmuch as the time course for stressor-provoked behavioral disturbances coincided with the locus ceruleus NE alterations, Weiss et al. (1981) suggested that this region may be fundamental in accounting for behavioral alterations. Essentially, it was suggested that a stressor may have resulted in a reduction of NE in the locus ceruleus, such that insufficient NE was subsequently released to produce autoreceptor stimulation. As a result, increased release of NE was elicited in forebrain terminal regions. As will be seen later, the supposition that locus ceruleus NE activity was associated with stressor-induced behavioral disturbances was supported by pharmacological manipulations of NE unit activity (Simson and Weiss, 1988; Weiss and Simson, 1985,1989).

In addition to its immediate effects, a stressor may proactively influence the NE response to a subsequently applied stressor. In particular, following exposure to an uncontrollable stressor of moderate severity, hypothalamic NE reductions persist for only a few hours. Upon subsequent reexposure to cues associated with the stressor, the utilization of NE is increased (Cassens et al., 1980), whereas reexposure to a stressor itself, even of limited severity, will readily reinduce the NE reductions (Anisman and Sklar, 1979; Irwin et al., 1986a; *see also* Rasmussen and Jacobs, 1986; Rasmussen et al., 1986).

The effects of stressors on NE activity and levels will not only vary with experiential factors, but also as a function of several organismic variables. For example, several investigators demonstrated that the NE variations associated with an acute stressor are dependent on the age of the organism. Ritter and

Pelzer (1978) reported that stressors, such as footshock or hyperthermia, produced more pronounced reductions of telencephalic and hypothalamic NE in 3-mo than in 7-mo-old rats. Moreover, the recovery of NE to nonstressed levels in 7-mo-old rats required 24–48 h, whereas in the younger animals, recovery was complete within 24 h. In contrast to these findings, Ida et al. (1982) reported that NE levels and utilization were both affected by immobilization, and that the extent of the change was comparable in 2- and 15-mo-old rats. It was subsequently determined, however, that NE alterations were more persistent in older (12 mo) than in younger (2 mo) rats. In particular, in 2-mo-old animals, a stressor (3 h of immobilization) provoked an increase of MHPG accumulation in all regions examined, which was evident immediately after stressor termination, but absent 6 h after the stressor session. In the 12-mo-old rats, however, increased MHPG accumulation was still evident 24 h after stressor termination in hypothalamus, amygdala, thalamus, pons + medulla, and midbrain (Ida et al., 1984).

In contrast to the NE reductions ordinarily associated with an acute stressor, amine levels will equal or even exceed control values following chronic stressor exposure (e.g., Irwin et al., 1986b; Kvetnansky et al., 1977; Roth et al., 1982; Weiss et al., 1975). It appears that, after repeated or prolonged exposure to an aversive stimulus, NE synthesis is appreciably increased, thereby preventing the NE depletion. Interestingly, on termination of a chronic stressor, the excessive utilization of NE may drop off, whereas the enhanced synthesis persists (*see* Thierry et al., 1968b). Indeed, it was reported that, immediately after a fifth footshock session, [^{3}H]NE accumulation formed from [^{3}H]tyrosine was increased by approx 35%, whereas 24 h after such treatment, the increase was in the magnitude of 55% (Kramarcy et al., 1984). The persistent augmentation of NE synthesis does not require that the stressor be severe. It was shown that, 24 and 48 h after a series of saline injections (coupled with handling), NE synthesis in rat hippocampal and cortical synaptosomes was increased (Birch et al., 1986), possibly as a result of desensitization of α-$_2$ and β-autoreceptors (*see* Stanford et al., 1984).

The NE reduction associated with an acute stressor may be prevented even if animals have previously been exposed to a chronic regimen involving a different form of aversive stimulation. In particular, it was reported (Adell et al., 1988) that, although acute immobilization (i.e., taping rear and forelimbs to mounts fixed to a board and limiting head movement by a loop) reduced NE in the hypothalamus, pons + medulla, and to a lesser extent hippocampus, repeated restraint (in plastic tubes for 1 h/d over 24 d) increased NE in frontal cortex, midbrain, and hippocampus. In rats exposed to chronic restraint and then challenged by acute immobilization, NE levels were higher than those of rats that received only immobilization, but lower than those of rats exposed to the chronic restraint regimen. These data are consistent with the view that a chronic stressor treatment results in an adaptation, but also suggests that such an effect may be less pronounced when a cross-stressor paradigm is employed.

It has been maintained that, as long as the stressor is present, the enhanced synthesis is essential for amine supply to be maintained, thereby permitting the organism to deal with the ongoing insult. The sustained increase in synthesis, coupled with the decline in utilization following stressor termination, assures that adequate amine supplies will be present in order for the animal to deal effectively with impending stressors. In effect, the sustained increase of amine synthesis is seen as an anticipatory response, with time between stressor sessions or termination of the stressor itself serving as the conditioned stimulus. Indeed, as seen in Fig. 1, it has been demonstrated that, in chronically stressed animals, reexposure to the stressor-related cues may increase levels of NE, as well as accumulation of MHPG, thus suggesting a compensatory increase in synthesis (Irwin et al., 1986a).

It has been our contention that the enhancement of NE concentrations following a chronic stressor is essential for the organism to deal effectively with environmental demands and may thus reduce the occurrence of behavioral pathology. It might be supposed that experiential or organismic factors, or variables associated with the stressor regimen that limit the course of the adaptation will increase the likelihood of behavioral pathology. For instance, it was shown that, when the stressor was presented on an unpredictable basis, the course of the adaptation was slower to develop (Anisman et al., 1986). Theoretically, as the

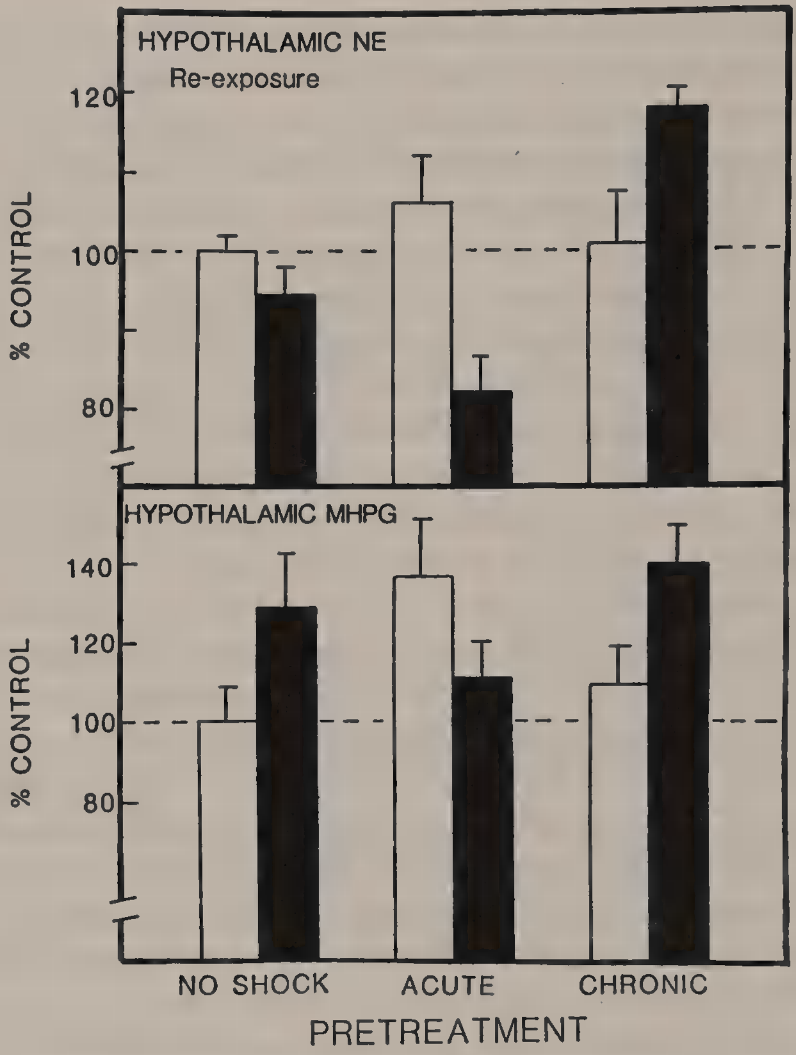

Fig. 1. Mean (±SEM) norepinephrine (NE) and MHPG concentrations in hypothalamus of mice that received either no shock, a single session of 360 shocks (2 s, 150 μA) (acute), or 14 sessions of 360 shocks on consecutive days (chronic). Two weeks afterward, mice were either placed in the apparatus and not shocked (open bars) or were exposed to 60 shocks (2-s duration) (closed bars). It will be noted that, in the acutely stressed mice, the shock reexposure treatment provoked a reduction of NE concentrations, whereas the same treatment applied to mice that had previously been exposed to a chronic stressor regimen resulted in enhanced NE concentrations (from Irwin et al., 1986).

predictability of the timing of the stressor or the type of stressor to which animals are exposed decreases, the neurochemical adaptation will be less likely to develop, and hence, the occurrence of behavioral disturbances will increase. Similarly, it might be expected that, in those animals in which the adaptation does not occur owing to organismic factors (e.g., strain or age of the organism), vulnerability to pathology will similarly be increased.

In assessing the adaptative changes associated with repeated stressor presentation, several investigators observed that chronic footshock or restraint produced a decrease in the density of β-NE receptors (e.g., Nomura et al., 1981; Stone, 1987; Stone and Platt, 1982; U'Pritchard and Kvetnansky, 1980), provoked increases in $\alpha\text{-}_2$ receptors (U'Pritchard and Kvetnansky, 1980), and increased $\alpha\text{-}_1$ receptor density (Lopez de Ceballo et al., 1983). Moreover, it has been determined that a chronic stressor regimen is associated with a reduction in the cAMP response to catecholamines (Stone, 1979, 1983; Stone et al., 1985). The reduction of β-NE receptor sensitivity and the downregulation of the cAMP response associated with a chronic stressor appeared to be independent of one another, since the stressor-provoked reduction of β-NE receptors in rat cortex was relatively transient, being absent 24 h following stressor termination (U'Pritchard and Kvetnansky, 1980; *see also* Stone et al., 1985), whereas the cAMP response was reduced to a comparable degree immediately and 24 h after a chronic stressor (Stone et al., 1985). It was reported that, in chronically stressed rats, the cAMP response to NE was reduced 24 h after stress, whereas the response to isoproterenol was marginally affected. Accordingly, it was suggested that the reduced cAMP response was associated with a reduction of nonβ-adrenergic receptors (Stone et al., 1985). It was subsequently demonstrated that a small decrease in β-adrenergic receptors in cerebral cortex and a larger reduction of α receptors were apparent 15–20 h after chronic restraint. The α receptors may potentiate the cAMP response to β-NE receptor stimulation, and thus the reduced NE-sensitive cAMP response associated with a chronic stressor may be a consequence of a reduction of α-adrenergic receptor density (Stone et al., 1986). These investigators have further suggested that adrenocorticotropin may contribute to the induction of the receptor alterations associated with a chronic stressor. More recently, it was reported that chronic stress may induce terminal sprouting in and around the area of the locus ceruleus, and that such an effect might be related to the development of β-receptor subsensitivity (Nakamura et al., 1989; *see also* Weiss and Simson, 1989).

Stone (1983) has maintained that, since a chronic stressor and repeated treatment with antidepressants both result in β-NE downregulation, the response to chronic stressors may reflect an adaptive change that limits the development of depressive symptoms. In accordance with this proposition, it was observed that disappearance of behavioral disturbances ordinarily associated with an acute stressor (i.e., immobility in a forced swim test and anorexia) occurred contemporaneously with the development of the receptor subsensitivity (Platt and Stone, 1982; Stone and Platt, 1982).

In considering the NE subsensitivity and the cAMP downregulation associated with antidepressant therapy, it should be made clear that such effects are probably not secondary to the blockade of NE reuptake, since these alterations are evident using atypical antidepressants (*see* Charney et al., 1981; McNeal and Cimbolic, 1986). It does seem, however, that there is a role for serotonin (5-HT) in the provocation of the receptor subsensitivity associated with antidepressant treatment. Intracerebral administration of the 5-HT neurotoxin, 5,7 dihydroxytryptamine, resulted in the elimination of the β-NE subsensitivity associated with desmethylimipramine treatment, but did not influence the downregulated cAMP response (*see* Janowsky and Sulser, 1987). The mechanisms underlying the chronic stressor-provoked β-NE subsensitivity have yet to be elucidated, although it does appear that, in rat hypothalamus, phospholipid metabolism may be involved (Torda et al., 1981). It remains to be determined, however, whether 5-HT activity plays a role in the development of the stressor-induced NE receptor subsensitivity.

2.2. Dopamine

The activity of central dopamine (DA) neurons, like that of NE, is appreciably affected by stressors. Moreover, there appear to be pronounced regional variations in this respect. For instance, stressors generally increase DA concentrations in whole hypothalamus, but when discrete nuclei are examined, a substantial DA reduction is evident within the arcuate nucleus

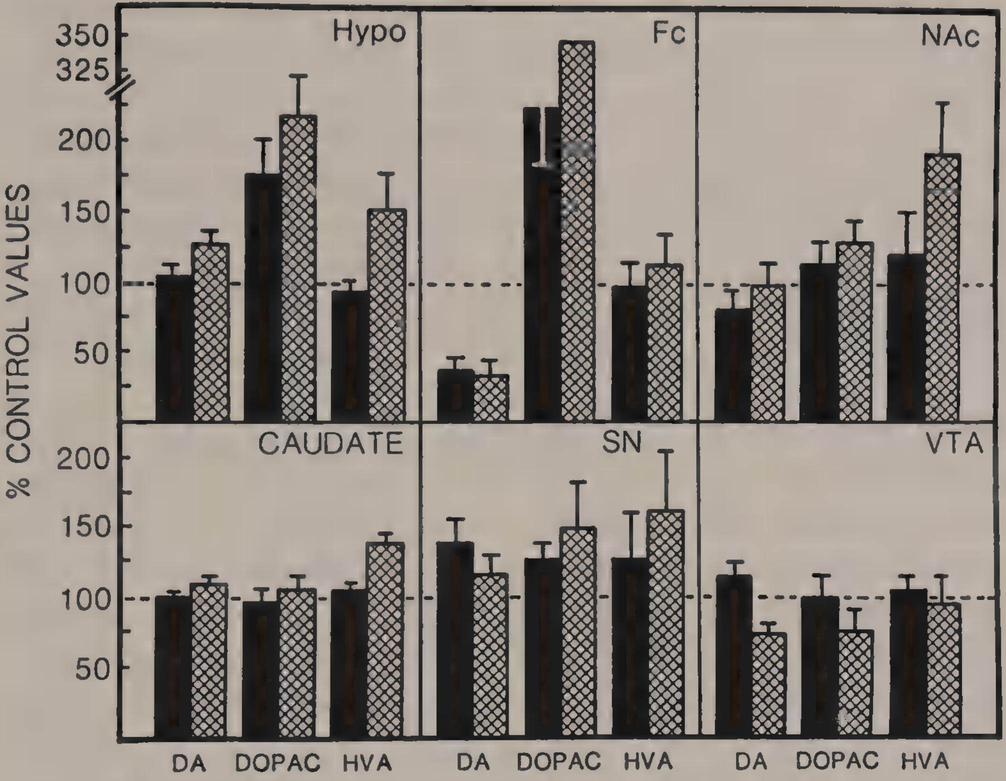

Fig. 2. Dopamine, DOPAC, and HVA as a percent of control values (±SEM) in the hypothalamus (Hypo), frontal cortex (Fc), nucleus accumbens (NAc), caudate, substantia nigra (SN), and ventral tegmentum (VTA) of mice that received either 30 or 360 shocks of 2-s duration (150 μA) (from Anisman and Zacharko, 1986).

(Kobayashi et al., 1976; Kvetnansky et al., 1976). In addition, as shown in Fig. 2, it was demonstrated that mesolimbic and mesocortical DA alterations were readily provoked by stressors. Typically, stressors increase the accumulation of the DA metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in the mesocortex and in the nucleus accumbens, and DA levels are reduced in the latter region (Claustre et al., 1986; Roth et al., 1988; *see also* Mantz et al., 1989). In contrast, stressors hardly affect DA concentrations or activity in the substantia nigra or in the caudate *(see* Anisman and Zacharko, 1986; Deutch et al., 1985; Herman et al., 1982; Herve et al., 1979; Thierry et al., 1976). Several investigators (Beley et al., 1976; Dunn and File, 1983; Heyes et al., 1988) have reported nigrostriatal DA alterations, but typically the stressor involved cold exposure (e.g., placement in a cold environment or immersion in cold water). Thus, these DA variations may be reflective of a thermoregulatory response rather than a genuine stressor effect.

The contribution of stressor controllability on DA neuronal activity has not been extensively reported. In our laboratory we observed that, within the mesocortex, DA reductions were as readily provoked by controllable footshock as by uncontrollable fooootshock. Moreover, this was the case irrespective of whether mice had received as few as 30 trials or as many as 360 trials (Anisman and Zacharko, 1986). In contrast, it was recently reported (Heinsbroek et al., 1989) that in rats uncontrollable footshock resulted in a greater DOPAC increase in mesocortex than did controllable footshock. Moreover, this effect was more pronounced in females than in males. Although the contribution of stressor controllability to the induction of DA variations remains to be resolved, the notion might be entertained that species and gender might contribute to the expression of the mesocorticolimbic DA alterations. It ought to be considered as well that anxiety associated with the stressor may contribute to the enhanced DA turnover. In particular, it was demonstrated that treatment with the anxiolytic, diazepam, or the β-NE inhibitor, chlorpropanol, which acts as an anxiolytic (*see* Fadda et al., 1978; Fekete et al., 1981; Laveille et al., 1978), prevented the stressor-provoked enhancement of mesocortical DOPAC.

As in the case of NE, it seems that a stressor may have long-term proactive repercussions. It was demonstrated, for instance, that presentation of cues that had previously been paired with a stressor effectively increased mesocortical DOPAC accumulation (Herman et al., 1982; Deutch et al., 1985). Moreover, several investigators reported that exposure to a stressor enhanced the subsequent behavioral effects of pharmacological treatments known to stimulate DA receptors (Antelman and Chiodo, 1983; Antelman et al., 1980; MacLennan and Maier, 1983). Thus, it appears that exposure to a stressor may have a persistent sensitizing effect on DA neuronal activity. Likewise, a stressor experience may influence the behavioral response to pharmacological manipulations affecting NE or 5-HT transmission (Anisman et al., 1985; Curzon, 1989).

The DA reductions elicited by acute stressors may be absent following a chronic stressor (Herman et al., 1984). Moreover, the DOPAC accumulation associated with a chronic stressor may be less pronounced than that associated with an acute stressor (Anisman and Zacharko, 1986; Herman et al., 1984). Kramarcy et al. (1984) reported that the increased mesocortical DA synthesis (and utilization) evident after acute shock is not present immediately or for 24 h following five sessions of footshock. Thus, the prevention of the DA reductions following a chronic stressor may be attributable to moderation of the excessive release of DA that is ordinarily engendered by an acute stressor.

2.3. Serotonin (5-HT)

Like NE and DA, the turnover and/or concentrations of 5-HT may be influenced by stressors (Joseph and Kennett, 1981; Kennett and Joseph, 1981); however, it appears that the stressor severity necessary to alter 5-HT utilization is greater than that required to engender NE alterations (Thierry et al., 1968a; *see also* Wilkinson and Jacobs, 1988). Moreover, regional differences exist with respect to the effects of stressors, such that increased concentrations of the amine are elicited by environmental insults in some brain regions (e.g., pons + medulla, septum, striatum), whereas decreases are apparent in other areas (midbrain, posterior hypothalamus) (Hellhammer et al., 1983). Exposure to various durations of immobilization stress (30–300 min), which did not affect 5-HT concentrations, resulted in increased 5-hydroxyindoleacetic acid (5-HIAA) accumulation, but this effect was more readily provoked in cerebral cortex and brainstem than in hippocampus, striatum, and diencephalon (Morgan et al., 1975). More recently, it was reported that a 15-min session of footshock increased accumulation of 5-HIAA and reduced concentrations of 5-HT in the mesocortex and in the hypothalamus of CD-l mice. When a more protracted stressor period was employed (30 min), amine utilization increased still further, as reflected by greater accumulation of 5-HIAA; however, 5-HT levels were comparable to those of nonstressed animals, whereas brain tryptophan levels increased appreciably

(Dunn, 1988). Thus, it was suggested that the increased availability of tryptophan increased synthesis of 5-HT, thereby replenishing the amine stores. Paralleling some of these findings, it was reported that in frontal cortex chronic restraint increased 5-HT and 5-HIAA, suggesting that the enhanced 5-HT concentrations likely stemmed from a compensatory increase in synthesis (Adell et al., 1988).

Several organismic variables may be identified that may influence the effects of stressors on 5-HT activity. In particular, cold exposure differentially influenced 5-HIAA accumulation in rats as a function of age (Algeri et al., 1982). The utilization of 5-HT was greater in 18- and 29-mo-old rats than in 4-mo-old animals. It will be recalled that the effects of cold exposure on catecholamine activity are quite different from those induced by other stressors, possibly reflecting a thermoregulatory response rather than a stressor effect *per se*. Inasmuch as older rats were also found to have greater difficulty in compensating for heat loss than younger animals, it is premature to dismiss the possibility that the age-related differences of 5-HT utilization in response to cold might likewise involve a thermoregulatory response. In addition to the age of the organism, it seems that genetic factors may contribute to the expression of the 5-HT variations associated with a stressor. We observed that footshock produced 5-HT and 5-HIAA alterations, the extent of which varied across several strains of mice (A/J, BALB/cByJ, C57BL/6J, DBA/2J, C3H/HeJ, CD-l) and the brain region examined (Shanks et al., 1988). It is conceivable that some of the divergent effects that have previously been reported may stem from the stressor severity employed or the strain of animal examined.

The contribution of stressor controllability on serotonergic changes has not been extensively assessed. Sherman and Petty (1980) reported that, within the lateral septum, uncontrollable shock more readily induced 5-HT reductions than did controllable shock. In contrast, controllable and uncontrollable shock did not differentially influence mesocortical 5-HT activity (Heinsbroek et al., 1989), whereas controllable shock was more likely to influence hypothalamic 5-HT concentrations (Weiss et al., 1981). It was suggested that the 5-HT variations observed in hypothalamus might reflect the effort associated with the act of emitting an escape response. At this point, unfortunately, there are simply too few data available to provide a definitive conclusion concerning the contribution of stressor controllability to 5-HT alterations.

2.4. Opiate Peptides

The effects of stressors are not restricted to the biogenic amines, but may influence opiate peptide activity, as well. In rats, acute stressors reduce pituitary β-endorphin, while increasing plasma levels of this opiate peptide (Guillemin et al., 1977; Knepel et al., 1983; Madden et al., 1977). Likewise, variations of plasma β-endorphin may be reported in humans following surgery (Owen and Smith, 1987), child birth (Pancherri et al., 1985), and electric footshock (Knepel et al., 1983). In addition to the activation of peripheral opioid mechanisms, stressors may influence the activity of various central neuropeptides (Pert, 1982). For instance, enkephalin availability in the hypothalamus was reduced by exposure to an acute session of uncontrollable footshock (Fratta et al., 1977; Rossier et al., 1978). Other stressors, including food deprivation and mild-tail pinch, decreased dynorphin activity in the cortex, whereas cold exposure produced a decline in hypothalamic dynorphin activity (Morley et al., 1982). Although stressors have a relatively transient effect on central neuropeptides, it seems that the opioids may be subject to conditioning-like effects, much like those described for NE and DA (Chance et al., 1978).

Like the stressor-provoked opioid variations observed in terminal fields, recent data suggest that alterations of neuropeptide activity occur at the site of origin of the major ascending NE and DA pathways. It was observed, for instance, that the conditioning or sensitization of locus ceruleus unit activity by stressor exposure was appreciably influenced by opioid manipulations (Abercrombie and Jacobs, 1988; Abercrombie et al., 1988; Morilak et al., 1987a,b). These findings are consistent with the demonstration of a dense distribution of opioid receptors (Atweh and Kuhar, 1977; Lewis et al., 1985), and high levels of opioid immunoreactivity in and around the area of the locus ceruleus (Finley et al., 1981; Leger et al., 1983). Moreover, inhibition of locus ceruleus unit activity has been demonstrated following central opioid administration (Bird and Kuhar, 1977; Guyenet and Aghajanian, 1979). It was suggested that stressors activated the locus ceruleus NE system, resulting in fear and/or anxiety, whereas coactivation of an endogenous opioid system, which functioned to inhibit the activity of the locus ceruleus, favored adaptive behavioral coping (Abercrombie and Jacobs, 1988; Abercrombie et al., 1988; Morilak et al., 1987a,b). In fact, it was recently demonstrated (Tanaka et al., 1989) that the increased MHPG associated with immobilization stress was attenuated by intraventricular Met-enkephalin administered prior to the stress session. When Met-enkephalin was administered after the stress session had begun, the accumulation of MHPG was not affected. These investigators also reported that the Met-enkephalin treatment was associated with an attenuation of stressor-elicited defecation and weight loss, thus supporting the contention that the opiate peptide played an essential role in the regulation of the NE response initially provoked by a stressor.

In addition to the modulating influence of opioids on central NE activity, stressor-induced alterations of central DA turnover can be influenced by opioid administration. In particular, both Met- and Leu-enkephalin, as well as their respective μ and δ receptors, may be identified in the ventral tegmental field (i.e., the VTA, the A10 region of the DA containing mesolimbic/mesocortical system) (Johnson et al., 1980; Moskowitz and Goodman, 1984). It was demonstrated that exposure to uncontrollable stressors augmented enkephalin activity in the VTA and accelerated DA turnover in mesocorticolimbic sites. Moreover, it appeared that intracerebral administration of enkephalin analogs into the midbrain tegmentum increased stressor-induced DA turnover in the nucleus accumbens and mesocortex (Kalivas et al., 1983). Conversely, the stressor-induced variations of DA activity from the mesocortex were prevented by the opioid antagonists, naloxone (Miller et al., 1984) and naltrexone (Kalivas and Abhold, 1987). Likewise, direct application of naltrexone into the A10 region attenuated the footshock-elicited elevation of DA metabolism (Kalivas and Abhold, 1987). Additionally, reduction of substance P, which appears to be colocalized in the tegmentum, attenuates mesocortical DA turnover engendered by uncontrollable footshock (Bannon et al., 1983). In contrast, Deutch et al. (1987) demonstrated that mild footshock increased neurotensin concentrations in the A10 region, and such an effect was associated with elevated levels of DOPAC in the mesocortex. There is also reason to suspect that sensitization/conditioning may be involved in enkephalin/neuropeptide interactions with central DA activity. Specifically, application of inescapable footshock potentiated the augmented DA turnover ordinarily elicited by administration of the enkephalin analog, D-Ala^2-Met^5-enkephalinamide (DALA) into the VTA, whereas naltrexone antagonized this effect. Likewise, prior administration of DALA resulted in the sensitization of the neurochemical response to a stressor (Kalivas et al., 1983, 1986). Paralleling this finding, it was demonstrated (Cabib et al., 1984) that the sensitivity to DA agonists was augmented in animals that had received repeated immobilization stress, an effect that was prevented by prior administration of naloxone. It was suggested that the acute stress-induced activation of enkephalin activity in the VTA served to release DA neurons from tonic inhibition. In animals exposed to a chronic stressor or repeated opioid administration, the disinhibition of somato-dendritic DA release is presumably reduced, provoking less stimulation of tegmental autoreceptors, thereby increasing DA release in terminal fields on reexposure to the stressor (Kalivas et al., 1988).

In addition to the effects of stressors on central opioid release, stressors may affect central opiate receptors. For example, Sirakova et al. (1988) reported that immobilization stress (i.e., 24 h coupled with food and water deprivation) enhanced δ opioid receptor binding, with little effect on μ receptor binding in rat whole brain. Inasmuch as food deprivation has been shown to reduce levels of β-endorphin in the hypothalamus (Gambert et al., 1981), it is unclear whether the opioid receptor alterations stemmed from the immobilization procedure, the deprivation schedule imposed, or a combination of the two.

3. Stress and the Immune System

Although central nervous system transmitters had traditionally been considered independent of the immune system, mounting evidence has accumulated that these systems may influence one another. Moreover, it appears that immune activity may be influenced by stressors, just as central transmitter activity is subject to modification by stressful events. Thus, the view has been offered that psychological factors may influence immunocompetence, thereby affecting vulnerability to pathology (Stein et al., 1976). As will be seen shortly, there is reason to suppose that immunological changes may also come to influence affective state, as well.

3.1. Stressor-Provoked Immunological Variations

Several investigators have demonstrated that, following exposure to an acute stressor, immune functioning was compromised. In particular, it was shown that in infrahumans, stressors may have had an immunosuppressive effect as evidenced by the inhibition of the T- and B-cell proliferative response in vitro (Keller et al., 1981; Monjan and Collector, 1977), suppression of natural killer (NK) cell activity (Shavit et al., 1983), and reduction of antibody titers and the plaque-forming cell (PFC) response following antigenic challenge (Esterling and Rabin, 1987; Okimura and Nigo, 1986). Moreover, it has been demonstrated that stressor exposure decreased resistance to viral infection (Chang and Rasmussen, 1965), delayed rejection of skin grafts (Wistar and Hildemann, 1960), and reduced the symptoms of autoimmune disorders (Grota et al., 1989). Likewise, in humans, bereavement and academic examinations are associated with immunosuppression, and such effects may be relatively long-lasting (Bartrop et al., 1977; Kiecolt-Glaser et al., 1984; Stein, 1981).

Only limited data are available concerning the role of coping factors in immune functioning. Shavit et al. (1984) reported that, although controllable shock was without effect, uncontrollable shock produced a suppression of the NK cell response in rats. Laudenslager et al. (1983) reported that uncontrollable shock inhibited lymphocyte proliferation in response to a T-cell mitogen, whereas controllable shock did not. Similarly, Mormede et al. (1988) demonstrated that, after ten stressor sessions, controllable and uncontrollable shock differentially influenced the response to the T-cell mitogen, phytohaemagglutinin (PHA), but not to Concanavalin A (Con A) or to Pokeweed mitogen. In contrast to these reports, however, Gamzu et al. (1984) reported that controllable and uncontrollable footshock differentially influenced the response to a B-cell mitogen, but not to a T-cell mitogen as had been reported by Laudenslager et al. (1983). Moreover, Gamzu et al. (1984) made particular note of the fact that, in their laboratory, the effects of the stressor manipulations were not particularly reliable for reasons they were unable to determine. Indeed, Maier and Laudenslager (1988) have recently reported an inability to replicate their earlier findings (i.e., those of Laudenslager et al., 1983), and suggested in a companion paper (Laudenslager et al., 1988) that evaluation of antibody titers, rather that lymphocyte proliferation, was most sensitive to the effects of stressors. However, experiments conducted in this laboratory (Zalcman et al., 1988a) that evaluated an orchestrated immune response (i.e., antibody titers and / or the PFC response) revealed that both controllable and uncontrollable footshock were equally effective in provoking an immunosuppression.

Several recent experiments indicated that, although stressors markedly influenced the PFC response and antibody titers, such an effect was dependent on when the stressor was applied relative to antigen administration. In particular, if animals were exposed to footshock 72 h after inoculation with sheep red blood cells (SRBC), a profound immunosuppression was evident; however, a stressor applied at other times yielded no effects (Zalcman et al., 1988a). Interestingly, recent experiments (Zalcman et al., 1990) revealed that in older mice the extent of the suppression induced by a stressor was more pronounced, and the stressor severity required to elicit this effect was appreciably reduced. Curiously, when the stressor was applied immediately after inoculation, the PFC response and antibody titers were actually increased. The possibility exists that the stressor may have affected T-suppressor cells, particularly in the older

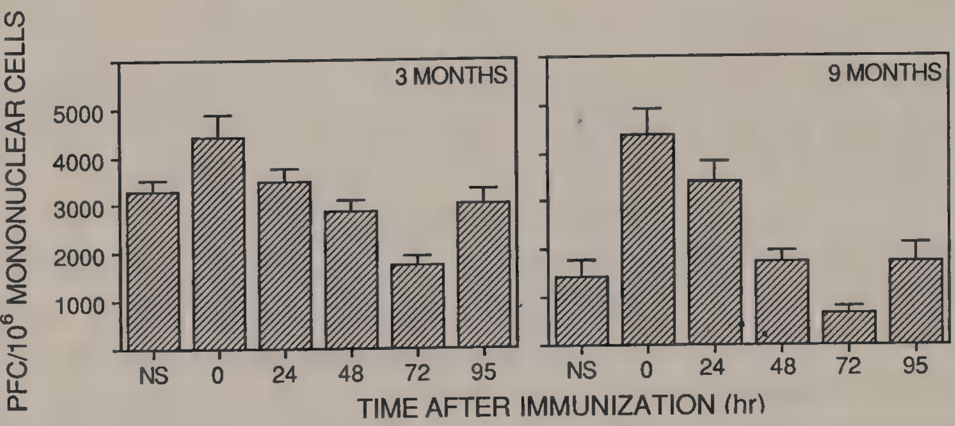

Fig. 3. Mean PFC response (±SEM) in (left) 3- and (right) 9-mo-old CD-1 mice 96 h after SRBC inoculation. Mice were exposed to footshock (360 shocks, 2-s duration, 150 μA) either immediately (0) or 24, 48, 72, or 95 h after immunization (from Zalcman et al., 1990).

animals, thus leading to an apparent enhancement of the immune response. It will be recognized, however, that the immunopotentiation may, in fact, be disadvantageous in that it may lend itself to enhanced autoantibody activity (*see* Fig. 3).

A series of experiments conducted by Ader and his associates (*see* Ader and Cohen, 1985) suggested that immune functioning may be subject to conditioning. In particular, it was demonstrated that exposure to saccharin some time after pairing of this novel taste with the immunosuppressant, cyclophosphamide, resulted in a reduction of the PFC response (Ader and Cohen, 1985). This treatment was also found to retard the rejection of skin grafts (Bovjberg et al., 1982) and to delay the symptoms of lupus erythematosus in an animal model of this autoimmune disorder (Ader and Cohen, 1982). Conditioning-like effects may also be apparent in a stress paradigm, in that exposure to cues that had previously been paired with shock reduced lymphocyte proliferation in response to the mitogens, PHA and Con-A (Lysle et al., 1988). Moreover, these investigators demonstrated that the extent of the suppression was reduced in animals that initially received the conditioned stimulus (CS) shock pairings and then were exposed to a series of CS presentations in the absence of shock (i.e., extinction). Paralleling the findings of Lysle et al. (1988), it was demonstrated that, in mice that had been exposed to footshock and then inoculated with SRBC 2 wk later, exposure to the shock apparatus (72 h after inoculation) engendered a marked suppression of the PFC response (Zalcman et al., 1989a). It is of particular significance, however, that if mice were initially exposed to footshock immediately after inoculation, then subsequent reexposure to the stressor 72 h later did not produce the immunosuppression, whereas reexposure to apparatus cues had the effect of increasing the PFC response and antibody titers. Thus, the possibility was raised that under the latter conditions (i.e., when stressor application occurred in close temporal congruity with SRBC inoculation) a compensatory conditioned immunoenhancement may be engendered. To be sure, this conclusion must be considered as being a highly provisional one; however, these data suggest that the effects of a stressor or cues associated with it are dependent on the organism's previous stress history, as well as the timing of the stressor relative to antigenic challenge.

Finally, as in the case of several of the central neurochemical consequences of stressors, it has been demonstrated that the immunosuppression ordinarily evident after acute shock may be absent after a chronic stressor (Folch and Waksman, 1974; Lysle et al., 1987; Monjan and Collector, 1977; Zalcman et al., 1988b). Likewise, the tumor-enhancing properties of acute uncontrollable stressors may have been abrogated by a chronic regimen where the stressor was applied at predictable intervals (Sklar and Anisman, 1981). It is conceivable that, if the regimen involves a series of different stressors applied at unpredictable times, then no such adaptation will be evident.

The findings that some components of the immune response may be influenced by stressor controllability and subject to conditioning effects provide *prima facie* evidence in favor of the notion that psychological factors, and hence central nervous system events, may contribute to immune activity. There are, in fact, ample data showing that electrolytic lesions of the anterior hypothalamus affect immune functioning (Cross et al., 1980, 1982, 1986; Roszman and Brooks, 1985; Macris et al., 1970) as will pharmacological manipulations of central catecholamines (Cross and Roszman, 1988; Cross et al., 1980, 1986; Hall and Goldstein

1981). Moreover, it appears that opioid peptides influence immune functioning (Johnson et al., 1982), although the ability to elicit such effects appears to vary with different neuropeptides (*see* Gilmore and Weiner, 1988).

3.2. Central Nervous System (CNS) and Immune System Interactions

The well-documented immunosuppressive effects of corticosteroids initially gave rise to the notion that the effects of stressors and of central neurotransmitter manipulations may be mediated by steroids (*see* Munck et al., 1984). However, studies involving adrenalectomized animals or inhibitors of corticosteroid synthesis made it clear that immune functioning may be affected by corticosteroid-independent mechanisms (e.g., Keller et al., 1983). There are several candidates that might act as a mediator between the CNS and immune system. Among others, various pituitary hormones, including ACTH, β-endorphin, prolactin, and growth hormone, are subject to neural modulation and their potential effects may thus be secondary to central neurotransmitter alterations. These pituitary factors could influence activated lymphocytes that bear cell surface receptors for a variety of neurotransmitters and hormones (Bourne et al., 1974; Hall and Goldstein, 1981; Smith et al., 1983; Wybran et al., 1979). Additionally, it has been demonstrated that primary and secondary immune organs received sympathetic innervation (Bulloch and Moore, 1981; Felten et al., 1981), and it has been suggested that CNS–immune system interactions may be mediated by surface receptors on lymphocytes, which traverse microenvironments richly innervated by autonomic fibers (Williams and Felten, 1981).

The relationship between immune activity and CNS functioning appears to be bidirectional. Not only will immune activity be affected by CNS manipulations, but it seems that the activity of central neurotransmitters will be altered by immunological manipulations. Inoculation with SRBC increased the turnover of hypothalamic NE, and such effects coincided with the time of peak immune activity (Besedovsky et al., 1977, 1983). In accordance with the findings of Besedovsky et al. (1983), it was reported that SRBC administration in mice resulted in hypothalamic NE reductions, which appeared to be restricted to the paraventricular nucleus (Carlson et al., 1987). Although the hypothalamic alterations were most notable at the time of the peak immune response (i.e., 4 d after inoculation), hippocampal NE and 5-HT alterations occurred considerably earlier (i.e., 2 d after immunization). We recently observed similar effects, but noted that NE reductions were also evident in the locus ceruleus, whereas in the nucleus accumbens, DA concentrations were reduced 3 d after immunization (which coincided with the day preceding the peak immunological response) (Zalcman et al., 1989b). Thus, it appears that some aspect of the immune response, which occurs relatively soon after antigenic challenge (e.g., macrophage and interleukin-1 activation), may be promoting these relatively early neurochemical alterations. In fact, it has been shown that systemic administration of the immune system messenger, interleukin-1 (IL-1), increases MHPG accumulation in the hypothalamus (Dunn, 1988; Kabiersch et al., 1988), brain stem, spinal cord, and forebrain (Kabiersch et al., 1988), as well as tryptophan in brainstem, hypothalamus, and prefrontal cortex (Dunn, 1988).

A specific role for IL-1 in immune–brain interactions is derived from the demonstration that systemic injection of this cytokine in mice provoked ACTH release in cultured pituitary cells (Woloski et al., 1985), and stimulated ACTH and corticosterone secretion (Besedovsky et al., 1975). It was suggested that the central action of IL-1 was mediated through the hypothalamus, since central application of IL-1 induced the secretion of CRF, which in turn stimulated ACTH release from the pituitary (Berkenbosch et al., 1987; Sapolsky et al., 1987). Furthermore, CRF release was enhanced without a concomitant release of other hypothalamic hormones and also occurred at subpyrogenic doses (Berkenbosch et al., 1987; Sapolsky et al., 1987). These data suggest that IL-1 can influence neuronal functioning, and that such an effect may occur through the adrenal–pituitary axis.

Antigen administration may have resulted in the production of neuroendocrine hormones, including ACTH and endorphins (Smith and Blalock, 1981), as well as thyrotropin (Smith et al., 1983). Moreover, the injection of Newcastle Disease Virus into mice resulted in a time-dependent increase in plasma corticosterone, which was also present in hypophysectomized animals, suggesting a pituitary-independent production of the steroid (Blalock, 1984). These investigators identified an ACTH-like substance in the spleen, indicating that the increase in corticosterone in the hypophysectomized mice was mediated though a "lymphoid–adrenal" axis. In contrast to its ability to transduce sensory stimuli, such as light or touch, the brain is only capable of recognizing the entry of foreign pathogens into the body through communication with the immune system. In effect, the view was offered that the immune cells may have acted in a sensory capacity to identify foreign matter and, being endowed with its own neuroendocrine capacities, could have communicated with other immune cells as well as with the CNS (Blalock, 1984; Blalock et al., 1985; Besedovsky et al., 1985).

It will be recognized that the hormonal and neurochemical consequences of antigenic challenge are reminiscent of the effects ordinarily elicited by stressors. Moreover, many of the immunological consequences of stressors are remarkably consistent with the neurochemical effects associated with such a treatment. As a working hypothesis it might be supposed that antigens, possibly through IL-1, act on endogenous processes much in the same way as stressors do (Dunn, 1988), and as such might ultimately engender behavioral effects like those produced by stressors. At present there is a dearth of information concerning the possibility that this is, in fact, the case. In their recent review of the literature, Dantzer and Kelley (1989) documented some of the behavioral effects of central administration of cytokines. Such treatment resulted in general malaise, decreased activity, social investigation, feeding and water intake, and weight gain, as well as changes in slow wave sleep pattern. It will be recognized that some of these symptoms are provoked by stressors, and also are characteristic of clinical depression. In light of the apparent reciprocal relationship between immune functioning and central neurochemical activity, it might be appropriate not only to consider the impact of psychological stressors on the affective state and the effects of psychological processes on immune functioning (e.g., the well-documented immunosuppression associated with depression and with grief), but also the potential contribution of immunological alterations on psychological processes.

4. Behavioral Consequences of Uncontrollable Stressors

4.1. Escape Disturbances

It will be recalled that, on exposure to a stressor, the defensive response styles of animals are restricted. If the response required of the animal is commensurate with these species-specific defensive behaviors, then the response will be acquired quickly. In contrast, when the response is one that animals are ordinarily not prepared to emit, acquisition will progress relatively slowly. Typically, if animals are exposed to escapable footshock, then subsequent performance in the same or in a different task progresses readily. However, animals that received an identical amount of inescapable footshock (applied in a yoked paradigm) will exhibit pronounced performance deficits in a subsequent escape task, characterized by repeated failures to escape the stressor. Accordingly, the suggestion was offered that the psychological dimension of coping behaviorally with the insult, as opposed to the aversive stimulation *per se*, was responsible for the behavioral disturbance (*see* Maier and Seligman, 1976). According to these investigators, the uncontrollability of stressor termination resulted in animals learning that their behavior and outcomes were independent of one another. As a consequence of this "learned helplessness," animals ceased attempts to escape or avoid, and passively accepted the shock. In accordance with this position, it was demonstrated that, if animals were initially trained to escape from a stressor, then the deleterious effects ordinarily provoked by an uncontrollable stressor were mitigated. In effect, learning that control over stressor termination was, in fact, possible limited the cognitive disturbances that might otherwise may be engendered. In addition to postulating the development of helplessness, it was suggested that uncontrollable stressors would result in motivational disturbances, as well as associative deficits involving response–outcome associations (Maier and Seligman, 1976).

According to a second view, the uncontrollable aversive events resulted in excessive utilization of one or more central neurotransmitters, which ultimately led to a reduction in the concentrations of these amines, and thus precluded adequate performance (Weiss et al., 1976a). Proponents of a neurochemical accounting for the interference effect have argued that, among other things, the central transmitter alterations would have resulted in a disturbance in the animal's ability to initiate active responses, as well as deficits in the ability to sustain vigorous responses for protracted periods (Anisman et al., 1978). Moreover, it was suggested that, because the stressor resulted in a decline in active responses, shock offset would often be associated with immobility. The adventitious reinforcement of such responses may promote their adoption in a subsequent test situation where the response necessary for successful escape is an active one, thereby provoking performance disturbances (Anisman et al., 1978; Glazer and Weiss, 1976a,b).

The performance deficits induced by inescapable shock are not limited to a shuttle or a lever press escape task, and are evident in paradigms involving different stressors, e.g., water escape (Irwin et al., 1980; Weiss et al., 1981). In rats and mice, however, the performance deficits are not apparent unless the task is a motorically demanding one (i.e., where an active response must be sustained for several seconds in order to escape from the stressor). For instance, if the task is a motorically simple one, such as a nose-poke, then the interference is not evident. In a relatively demanding shuttle test, the interference may be apparent, and becomes more pronounced as the height of the hurdle is increased (Glazer and Weiss, 1976a,b) or when escape is briefly delayed, thereby requiring the animal to sustain an active response for several seconds before escape is possible (Anisman et al., 1978). Likewise, in a water escape task, the interference effect becomes more pronounced as the water temperature is reduced, a treatment that increases energy expenditure (Irwin et al., 1980).

When the behavior of animals exposed to inescapable shock was monitored on a second-by-second basis, Anisman et al. (1978) reported that upon shock onset mice initially exhibited high levels of activity, which declined markedly over the course of a 6-s shock. With repeated shock trials, the initial excitation persisted; however, the rate of the decline of shock-elicited activity became progressively more pronounced. Thus, when the response required of the animal was not motorically demanding or could be accomplished fairly quickly, the initial burst of excitation favored proficient escape responding. In contrast, when the task was a demanding one, or when the response could be achieved only after several seconds of shock, the reduced levels of motor activity favored the expression of the escape deficits. In effect, these data suggest that inescapable shock limits the animal's ability to sustain an active response, hence leading to behavioral disturbances (Anisman et al., 1978).

Whether behavioral disturbances will be evident after inescapable shock is dependent on the stressor parameters employed, as well as the time following the stressor session at which animals are tested. For instance, Glazer and Weiss (1976a,b) reported that escape deficits were apparent in rats tested soon after brief-duration, high-intensity shock, but were absent 24 h later. In contrast, long-duration, moderate-intensity shock provoked long-term shuttle deficits, but curiously no such disturbance was evident soon after the inescapable shock session. Likewise, in mice, Anisman et al. (1978) reported that escape deficits became more pronounced over the 24-h period following exposure to moderate severity shock. It was suggested (Glazer and Weiss 1976a,b) that two forms of the escape interference may have existed. The short-lasting interference was ascribed to a transient reduction of central NE, whereas the long-term interference induced by moderate severity, long-duration shock was attributed to the development of learned competing motor responses.

In order to account for the absence of an interference effect soon after exposure to moderate-intensity inescapable shock, Glazer and Weiss (1976b) and Anisman et al. (1978) suggested that high levels of anxiety engendered by the stressor may have prevented expression of motor disturbances or competing response tendencies that might otherwise have favored the interference effect. In accordance with this view, Prince and Anisman (1984) reported that, 24 h after exposure to inescapable shock, mice placed in a forced swim task exhibited decreased swimming (increased floating), whereas escapable shock was without effect. In contrast, immediately after stressor exposure, a motor excitation was seen, such that mice exhibited increased swimming relative to nonshocked animals. Unlike the response inhibition evident 24 h after stressor application, the motor excitation was provoked regardless of whether the stressor was controllable or uncontrollable. Moreover, consistent with the view that the motor excitation was related to anxiety, this behavior was eliminated by diazepam (Prince et al., 1986).

Since uncontrollable stressor application influences NE, DA, 5-HT, acetylcholine (ACh), and endorphin activity, it was argued that it was premature to ascribe the interference effect solely to the NE variations engendered by the stressor (e.g., Anisman et al., 1981b). Indeed, pharmacological manipulations that influenced central transmitters had predictable effects on escape performance that were reminiscent of those provoked by an uncontrollable stressor. Treatment with agents that reduced DA and/or NE synthesis (i.e., α-methyl-*p*-tyrosine [α-MpT] and FLA-63, respectively) or blocked DA receptors disrupted escape performance. However, this disruption was only apparent when the task necessitated that the active response be sustained for several seconds before escape was possible. If escape could be accomplished within 2 s of shock onset, then the drug treatments were without effect (Anisman et al., 1979). Likewise, increasing ACh levels by inhibition of acetylcholinesterase (i.e., following administration of physostigmine) disrupted shuttle escape performance (Anisman et al., 1981a). Predictably, treatments that increased catecholamine concentrations or blocked ACh receptors (L-DOPA and scopolamine, respectively) effectively eliminated the escape interference engendered by inescapable shock (Anisman et al., 1979,1981a). Parenthetically, the effects of L-DOPA and scopolamine could be distinguished from one another. Whereas L-DOPA was effective in antagonizing the interference effect irrespective of whether it was applied prior to inescapable shock or prior to testing, scopolamine treatment was only effective when administered prior to testing.

Evidence in favor of a role for 5-HT in provoking the interference effect was derived from several studies that revealed that treatment with the 5-HT antagonist, methysergide, mimicked the effects of inescapable shock (Hamilton et al., 1986), whereas acute systemic treatment with the 5-HT releasing agent, *p*-chloroamphetamine (Hamilton et al., 1976), or administration of 5-HT into the septum (Sherman and Petty, 1980) eliminated the escape interference. In contrast, intraventricular administration of the 5-HT neurotoxin, 5,7-dihydroxytryptamine, neither influenced the escape deficits associated with inescapable shock nor modified the effects of repeated treatment with antidepressants that affected the 5-HT system (Soubrie et al., 1986). It should be underscored as well that the differential effects of escapable and inescapable shock on 5-HT activity or levels have only been noted within the lateral septum (Petty and Sherman, 1982; Hellhammer et al., 1983), and at present there is still insufficient evidence to make a strong case for a primary role for 5-HT in subserving the stressor-provoked behavioral disturbances (*see* Weiss et al., 1981).

In their critique of the neurochemical accounting for the interference effect, Maier and Seligman (1976) questioned the validity of this model given that the neurochemical changes engendered by an acute stressor were relatively transient, whereas the behavioral disturbances produced by inescapable shock may be long-lasting. As indicated earlier, however, it was demonstrated that some of the neurochemical changes associated with a stressor may be subject to conditioning or sensitization, such that on reexposure to a stressor the amine reductions may be readily reestablished (e.g., Anisman and Sklar, 1979; Herman et al., 1982). Thus, during the early test trials in a shuttle task, the amine alterations were quickly reinduced and the interference effect emerged.

4.2. Vigilance and Anxiety

In addition to the escape interference elicited by an uncontrollable stressor, Maier and Seligman (1976) suggested that uncontrollable aversive events may have resulted in associative deficits wherein animals appeared less able to form response–outcome associations. It was reported that rats exposed to inescapable shock subsequently exhibited retarded acquisition of a response–choice discrimination (Jackson et al., 1980). In later experiments, it appeared that such a deficit was not evident unless the experimental situation contained irrelevant cues. Accordingly, the view was offered that uncontrollable stressors disrupted attentional processes, so that animals did not discriminate adequately between relevant and irrelevant stimuli (Minor et al., 1984). As an alternative to this position, Anisman et al. (1984) suggested that exposure to inescapable shock resulted in a perseverative response style, such that animals persisted in adopting response strategies that had previously been acquired or those innate defensive strategies that were highest in their response repertoires (Szostak and Anisman, 1985). Accordingly, animals may encounter difficulty in acquiring those response–outcome associations incompatible with response styles to which they are predisposed.

It will be recalled that Weiss and Simson (1985) proposed that uncontrollable footshock resulted in diminished NE levels within the locus ceruleus. Consequently, there would be less NE available for release, and hence, stimulation of autoreceptors on NE cell bodies within the locus ceruleus would be reduced. The reduced NE autoreceptor activity, in turn, would result in increased NE activity in terminal regions. Thus, although stressors may influence NE activity in several brain regions, the locus ceruleus is fundamental to the hypothesis advanced by Weiss and Simson (1985). These investigators noted that activation (disinhibition) of the locus ceruleus may have affected the processing of external information such that attention to irrelevant stimuli would be reduced, while increasing attention (vigilance) to those stimuli relevant to current environmental demands (Aston-Jones and Bloom, 1981; Aston-Jones et al., 1984; Foote et al., 1983). Weiss and Simson (1985) suggested that the vigilance discussed by Aston-Jones and Bloom (1981) may be related to anxiety, associated with activation of the locus ceruleus (*see* Redmond, 1987). This view is consistent with the findings that uncontrollable stressors engender both a response excitation and perseveration of response styles high in the animal's repertoire (Anisman et al., 1984; Prince and Anisman, 1984). In effect, although this position assumes a canalization of response styles (i.e., enhanced attention and responsivity to specific environmental cues), the view advanced by Maier and his associates (Jackson et al., 1980; Minor et al., 1984) asserts that the stressor results in the inability to differentiate relevant from irrelevant stimuli.

4.3. Anhedonia

In developing an animal model of depression, the behavioral disturbances seen in animals ought to be reminiscent of the symptoms that characterize the human disorder. To be sure, it has been demonstrated that stressors may induce sleep disturbances and decreased food intake, at least for short periods of time (*see* Weiss and Simson, 1985). In addition, there is reason to suppose that stressor application may engender an anhedonia, which is one of the primary symptoms of depression. Several approaches may be employed to evaluate the anhedonia that may stem from stressor application. For instance, given a choice between water and saccharin, animals ordinarily prefer the latter. However, in rats that had been exposed to a regimen involving a series of different stressors, this preference was reduced or eliminated (Willner, 1985). We have evaluated the anhedonic effects of environmental insults by assessing the effects of stressors on responding for electrical brain stimulation (ICSS) from various brain regions. It was observed that escapable footshock did not affect self-stimulation performance, whereas an equivalent amount of inescapable shock produced marked reductions of responding for ICSS. However, such an effect was dependent on the brain region supporting self-stimulation. In particular, as shown in Fig. 4, responding for brain stimulation from the nucleus accumbens or medial forebrain bundle was appreciably retarded in animals exposed to inescapable shock, whereas responding from the substantia nigra was unaffected (Zacharko et al., 1983a). These findings paralleled the observations that uncontrollable stressors modified mesolimbic DA

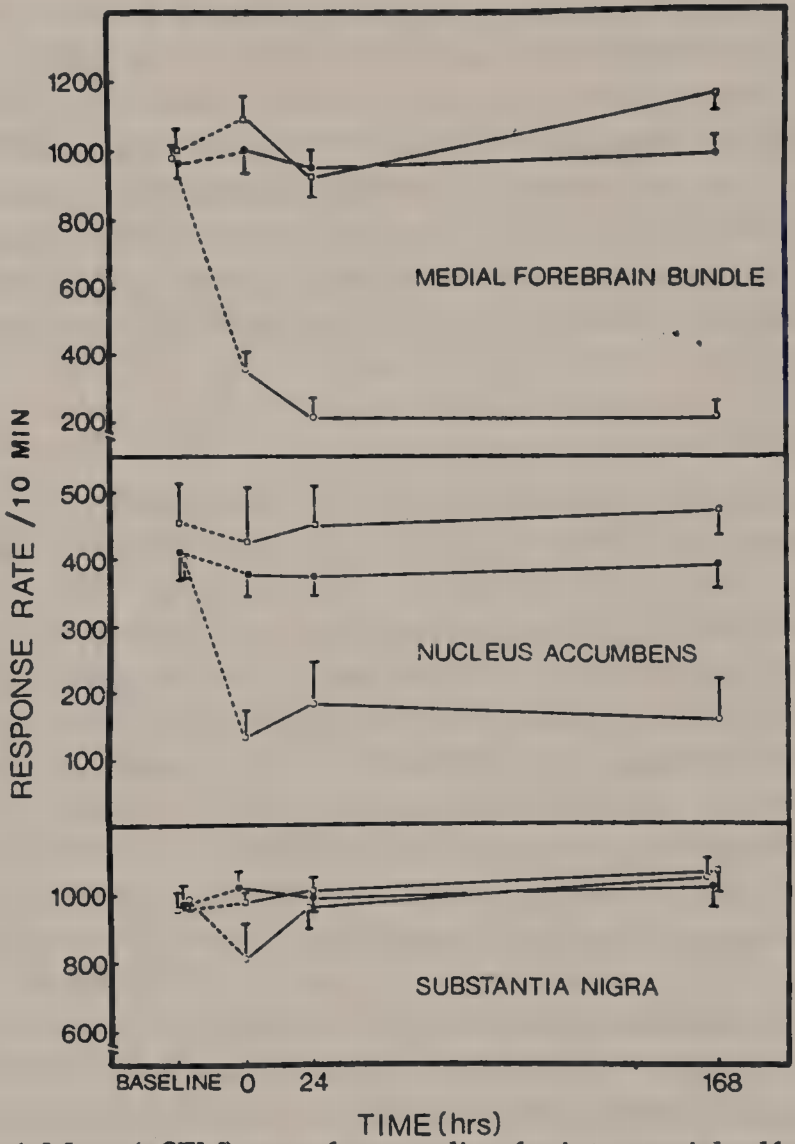

Fig. 4. Mean (±SEM) rate of responding for intracranial self-stimulation from the medial forebrain bundle, nucleus accumbens, or substantia nigra in mice that had been exposed to escapable shock (●), yoked inescapable shock (○), or no shock (☐). Baseline rates represent rate of responding on the day prior to shock treatment. Mice were tested immediately (0), and again 24 and 168 h after the shock session (from Zacharko et al., 1983a).

turnover and/or levels, without affecting nigrostriatal DA activity. Thus, it was provisionally suggested that stressors influenced motivational processes involving DA mechanisms. Of course, this does not imply that self-stimulation and its disruption by a stressor do not involve other transmitter systems.

The disruption of self-stimulation induced by inescapable shock is not restricted to DA terminal regions or to fibers of passage. Indeed, exposure to an uncontrollable stressor may induce marked reductions of reponding for ICSS from the ventral tegmentum (Kamata et al., 1986; Kasian et al., 1987). However, it seemed that the performance disturbances varied across different portions of the tegmentum (Kasian et al., 1987; Zacharko et al., 1990a). Although comparable baseline rates of responding for ICSS were evident from the ventral and the dorsal VTA, inescapable shock only disrupted responding from the dorsal A10 area (*see* Fig. 5). These data provide a functional differentiation of different portions of the VTA, commensurate with the neurochemical and electrophysiological delineation of the VTA reported by Deutch et al. (1985), suggesting that the tegmental field is not uniformly affected by stressors.

It is unlikely that the disruption of self-stimulation is the result of motoric disturbances. In order to minimize the influence of such a factor, the operant employed in the preceding studies was one of head-dipping, a response that is readily acquired, easily maintained, and not prone to disruption by treatments that influence motor activity (Bowers et al., 1984). Additionally, if the deficits of responding were the result of motoric factors engendered by the shock treatment, then it may be expected that ICSS rates would be disrupted irrespective of the brain region supporting self-stimulation. It will be noted, as well, that the shock-provoked disruption of self-stimulation was not peculiar to the particular stimulation parameters employed. Specifically, the reduction of responding for ICSS from the nucleus accumbens, medial forebrain bundle, and the dorsal VTA were evident when a descending current intensity paradigm was employed (Bowers et al., 1987; Kasian et al., 1987). In this paradigm, animals are initially trained to respond for ICSS at a current intensity sufficient to elicit moderate rates of responding. Animals were then given a baseline training session where the current was systematically reduced in 10-μA steps every 3 min over the course of a 30-min session. As depicted in Fig. 5, on subsequent exposure to inescapable shock, responding for brain stimulation at a wide range of the higher current intensities was

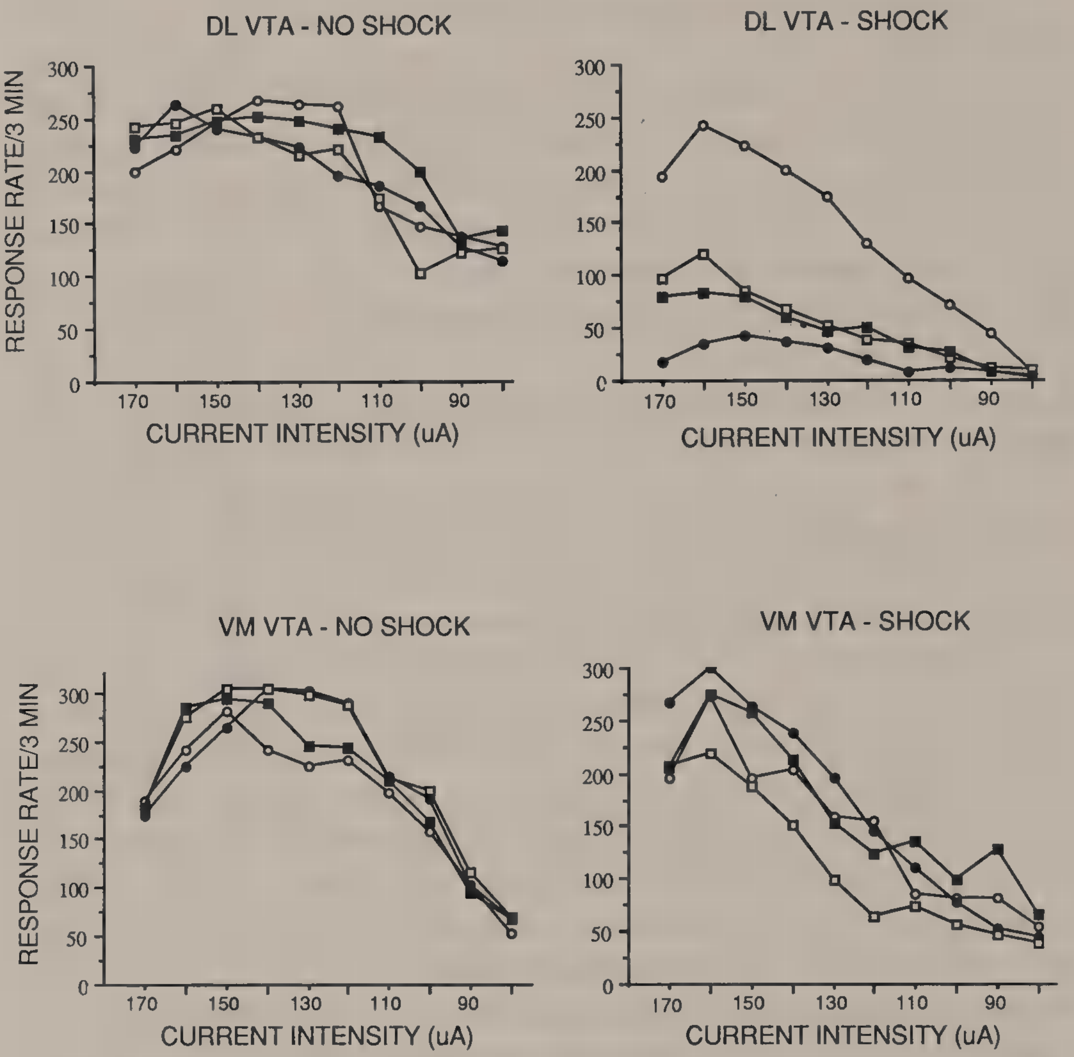

Fig. 5. Mean rate of responding for electrical brain stimulation from either the ventral (VM) or dorsal (DL) aspects of the VTA. Baseline rates of responding (–○–) are provided, as well as performance at each of three intervals (0[–●–], 24 [–□–], and 168 [–■–] h following exposure to either inescapable footshock or no shock. Mice were tested in a descending (170–80 μA) current intensity paradigm, in which animals were permitted to respond for 3 min at each current intensity, which decreased in 10-μA steps over the course of the 30-min test session (from Kasian et al., 1987).

reduced. At the lower intensities, the response reductions were less pronounced, probably owing to a floor effect.

Although the neurochemical changes induced by stressors are relatively transient, the stressor-induced disruption of self-stimulation may be long-lasting. However, it appears that the course of the disruption is dependent on the specific test schedule employed. Typically, when placed in the test chamber immediately following exposure to a stressor, animals display vigorous responding for brain stimulation, after which response rates taper off dramatically, often ceasing entirely. When returned to the test chamber several days afterward, animals initially display a short burst of responding followed shortly thereafter by the cessation of head-dipping for ICSS. Interestingly, if animals are exposed to a stressor and then are tested for the first time several days afterward, responding for ICSS is unaffected by the stressor experience. In effect, a long-term behavioral deficit may be induced by the stressor provided that animals have initially been tested soon after the first stressor experience. We have not determined why it is necessary for animals to have the experience in the task soon after inescapable shock in order for the long-term deficit to appear. It is possible that, soon after exposure to a stressor, the reduced DA levels limit the rewarding value of electrical brain stimulation leading to extinction of the response. It is conceivable that a conditioned DA change occurs on reexposure to the apparatus or that responding for brain stimulation after inescapable shock is aversive, and hence, long-term behavioral effects are manifested.

In accordance with the proposition that the self-stimulation disturbances associated with inescapable shock may be useful as an animal model of depression, the deficits of responding for ICSS from the nucleus accumbens were antagonized by repeated treatment with desmethylimipramine (DMI) (Zacharko et al., 1984a,b). It will be noted that, although DMI affected NE reuptake to a greater extent than it did other transmitters and the antidepressant qualities of this agent were usually ascribed to downregulation of β-NE activity, a positive effect was obtained despite the fact that the nucleus accumbens was a DA terminal region. Thus, these data support the contention that DMI may directly or indirectly influence DA mechanisms (Fibiger and Phillips, 1981).

5. Behavioral Adaptation Following Chronic Stressors

The NE and DA alterations associated with acute insults may be absent following repeated exposure to a stressor. The levels of the amine may rise, and downregulation of β-NE receptors may be provoked. In a like fashion, some of the behavioral changes exerted by acute stressors may not be expressed following chronic stressor application. In assessing the shuttle escape deficits associated with acute inescapable shock, Glazer and Weiss (1976b) noted that, with chronic stressor application involving relatively intense shock of brief duration, the escape deficits were absent. Thus, it was argued that the behavioral adaptation was likely attributable to the enhanced NE synthesis and levels that were engendered by repeated exposure to a stressor.

As indicated earlier, following chronic stressor application, the DA reductions ordinarily elicited by a stressor may be precluded (Herman et al., 1984). Paralleling these findings, we observed that the disruption of self-stimulation from the nucleus accumbens, as well as from the VTA, was eliminated following repeated exposure to footshock in the CD-1 mouse (Kasian and Zacharko, 1989; Kasian et al., 1989; Zacharko et al., 1983b). Once again, it appears that the behavioral and neurochemical consequences of a stressor may be congruent with one another. Interestingly, the adaptation was not apparent when testing was conducted in either a shuttle escape task or in a forced swim paradigm (Zacharko et al., 1983b). Although it was conceivable that the stressor parameters favored the development of learned competing response tendencies that were not subject to adaptation (cf. Glazer and Weiss, 1976b), it was unlikely that such competing response tendencies were responsible for the lack of an adaptation in the forced swim paradigm. Specifically, the characteristics of the shock and swim situations, as well as the response styles that could be adopted in the two tasks were markedly different from one another. It is our contention that the various behavioral consequences associated with stressors may be subserved by different neurochemical mechanisms and subject to differential rates of adaptation. As will be seen later, several of the behavioral consequences of stressors are independent of one another, and manipulations that affect one behavior may be ineffective in altering other behaviors.

It is important to emphasize at this juncture that the adaptation of self-stimulation associated with chronic footshock does not occur in all animals. In particular, in some mice, the adaptation does not occur, or alternatively, some indications of an adaptation are apparent after which performance again decays. In those animals in which the adaptation did not occur, repeated treatment with DMI augmented performance. In contrast, in animals in which a behavioral adaptation was apparent after repeated shock, the DMI treatment provoked a disruption of ICSS performance (Zacharko et al., 1983b). Thus, the suggestion was offered that, following a chronic stressor regimen, adaptive neurochemical changes occurred, thereby eliminating the stressor-induced anhedonia, as reflected by the high levels of self-stimulation. In a subset of animals, however, the chronic stressor regimen did not necessarily lead to the neurochemical and behavioral adaptation, and antidepressant therapy may be necessary for the expression of self-stimulation.

6. Genetic Differences in Response to Stressors

The symptoms that characterize a depressive disorder may vary appreciably across subjects, and it appears that the disorder may be biochemically heterogeneous. Indeed, pharmacological treatments of depression that are effective in one individual may be ineffective in a second. In a like fashion, it seems that considerable interindividual and interstrain differences exist with respect to the behavioral effects of acute uncontrollable stressors. As shown in Fig. 6, the shuttle escape deficits induced by inescapable shock vary considerably across strains of mice. For instance, exposure to inescapable shock provoked an appreciable retardation of shuttle escape performance in BALB/cByJ and C3H/HeJ mice, and a moderate disruption in C57BL/6J, A/J, and CD-1 mice, but had a small effect on DBA/2J mice (Shanks and Anisman, 1988). Likewise, the effect of stressors on ICSS from the nucleus accumbens varied across strains. Of particular interest was the finding that the disturbances

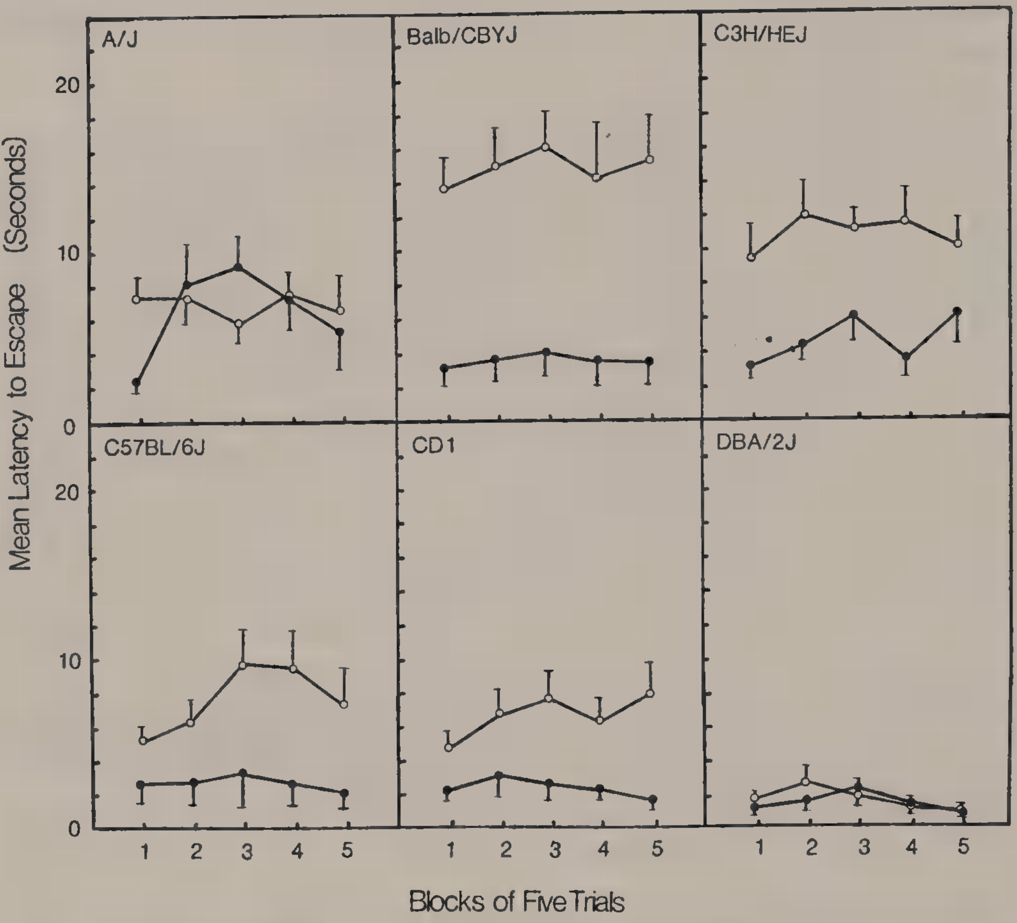

Fig. 6. Mean (±SEM) escape latencies over blocks of five trials in each of six strains of mice that had been exposed to either inescapable shock treatment (○—○) or no shock (●—●) 24 h earlier (from Shanks and Anisman, 1988).

seen in a shuttle test were unrelated to the disturbances seen in ICSS. In particular, as depicted in Fig. 7, inescapable shock disrupted ICSS in DBA/2J mice, had no effect in C57BL/6J animals, and actually enhanced responding in BALB/cByJ mice (Zacharko et al., 1987). Interestingly, a very different strain profile is evident in mice responding to brain stimulation from the mesocortex. In DBA/2J mice, inescapable footshock provoked a long-lasting retardation of ICSS, whereas in BALB/cByJ mice, a transient reduction of responding was produced, being apparent only when animals were tested immediately after stressor exposure (Zacharko et al., 1990b). The strain-specific alterations in ICSS could be readily differentiated from those seen in the shuttle test. Uncontrollable shock hardly affected shuttle performance in

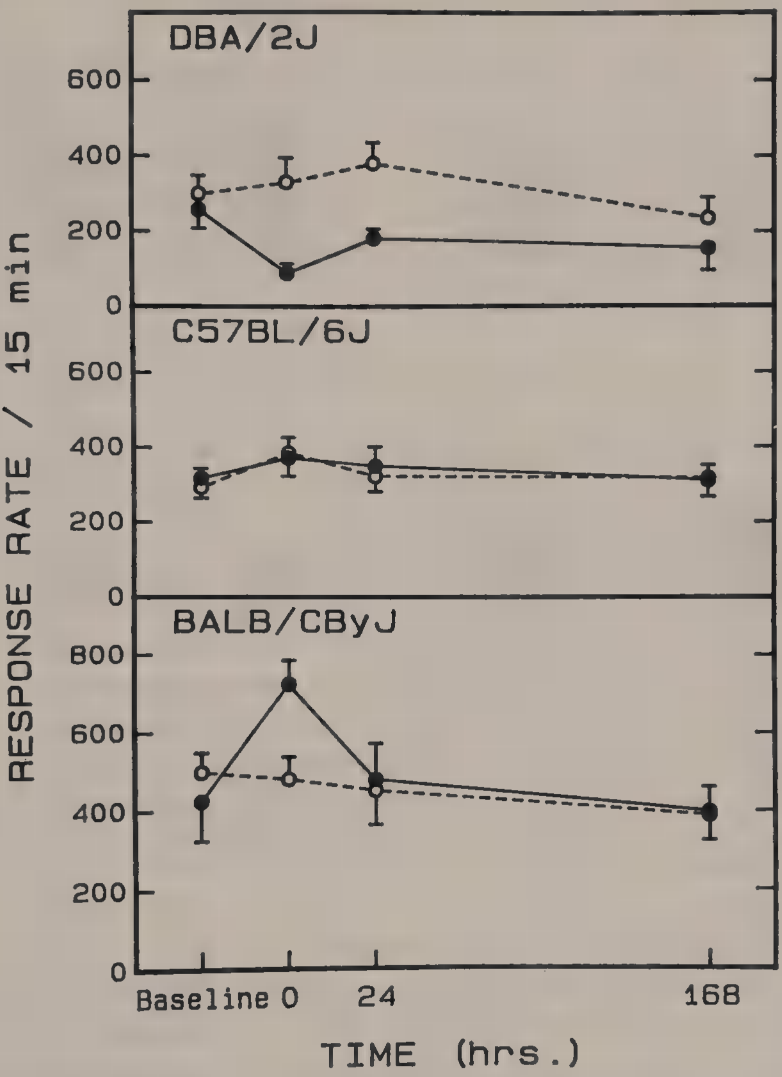

Fig. 7. Mean (±SEM) rate of responding for electrical brain stimulation from the nucleus accumbens in three strains of mice. Animals received a 15-min test session prior to treatment (baseline) and again immediately 24 h and 168 h following exposure to either inescapable footshock (●—●) (360 shocks, 2-s duration, 150 μA), or no shock (○—○) (from Zacharko et al., 1987).

DBA/2J mice, but produced a marked retardation of escape responding in the BALB/cByJ strain. Thus, a particular strain is not generally vulnerable or invulnerable (hardy) to the action of a stressor, but rather the impact of a stressor will be dependent on the specific behavioral task in which animals are tested. Similarly, within a given strain, vulnerability to the effects of a stressor on ICSS is region-specific. It is our contention that the array

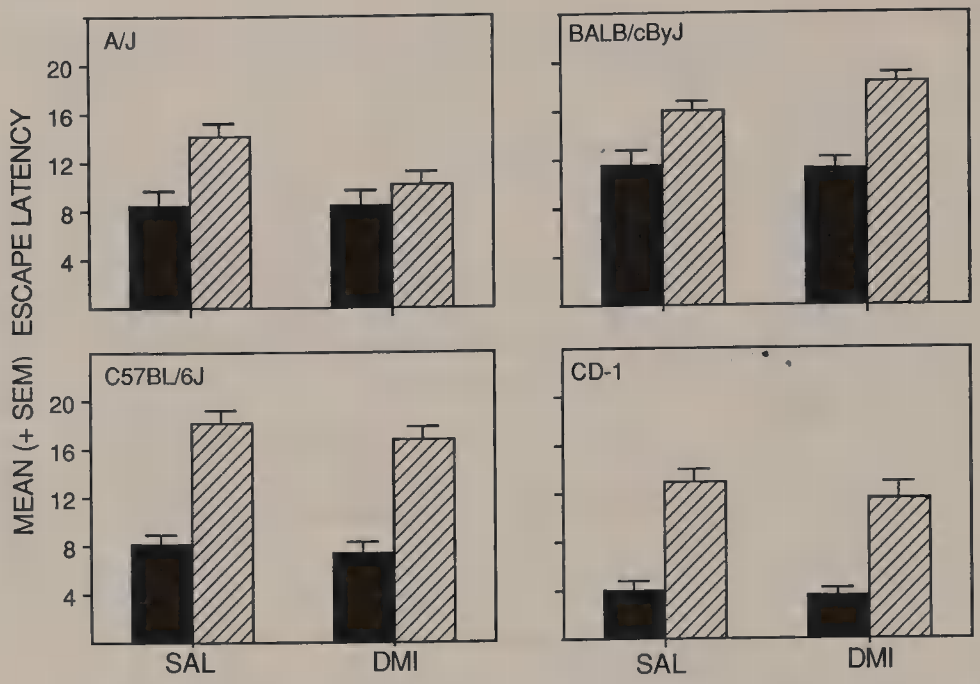

Fig. 8. Mean (±SEM) escape latencies (s) in four strains of mice that had been exposed to inescapable shock (☐) (360 shocks, 2-s duration, 300 μA) or no shock (■), and tested in a shuttle escape task 15 d later. Mice had received either saline or desmethylimipramine (DMI: 5 mg/kg, twice per day) for 14 d, and tested 1 h after the final drug treatment (from Shanks and Anisman, 1989).

of behavioral deficits that are engendered by a stressor are related to the neurochemical and hormonal disturbances associated with the environmental insult. The heterogeneity in response to stressors is, in a fashion, reminiscent of the presumed heterogeneity concerning the biochemical substrates of depression. The fact that inescapable shock differentially affects the strain profiles for ICSS from the mesocortex and nucleus accumbens also raises the possibility that different motivational processes may be affected by stressors. Although admittedly speculative, this hypothesis may account for the diversity of symptoms ordinarily seen across depressed individuals.

As in the case of human depression, where the efficacy of antidepressant treatments varies across individuals, the effectiveness of such treatments in modifying stressor-provoked behavioral deficits also varies across mouse strains. As shown in Fig. 8, the escape disturbances induced by inescapable shock in A/J mice was eliminated by repeated treatment with DMI. In contrast, the drug was ineffective in ameliorating the behavioral disturbance in either the BALB/cByJ, C57BL/6J, or CD-1 strain. In contrast, repeated bupropion treatment marginally reduced the deficit in the CD-1 mouse, but did not affect performance in the other strains. Finally, repeated treatment with amitriptyline reduced the deficits in A/J and CD-1 mice, although acute treatment was also effective in this respect (Shanks and Anisman, 1989).

In contrast to the effects of the antidepressants in the shuttle escape test, repeated DMI treatment eliminated the deficits of ICSS from the nucleus accumbens in the CD-1 mouse strain (Zacharko et al., 1984b). Conversely, we have observed that repeated treatment with neither DMI nor amitriptyline antagonized the disturbances of self-stimulation in DBA/2J mice from either the accumbens or the mesocortex (Baillie et al., 1988). Taken together, it would appear that in animals antidepressants only influence a subset of the symptoms comprising the depressive syndrome, and the specific symptoms affected by antidepressants vary with the strain of mouse examined.

Given the aforementioned strain differences in response to stressors and drug treatments, it is not surprising that environmental insults also induce strain-dependent neurochemical changes (Cabib et al., 1988a,b; Tassin et al., 1980). Although stressors generally influence NE and DA turnover, it seems that some strains of mice are relatively more vulnerable to such alterations than are other strains. It is important to emphasize that the strain-dependent amine variations also vary with the brain region examined. For instance, in some strains (e.g., C57BL/6J), NE reductions are readily induced in the locus ceruleus and in the hypothalamus, whereas in other strains (BALB/cByJ and C3H/HeJ), NE alterations are readily induced in frontal cortex and hypothalamus, but not in the locus ceruleus. Similarly, both the A/J and BALB/cByJ mice show marked alterations of DA activity in the nucleus accumbens, whereas in the mesocortex, the DA activity is markedly enhanced in A/J, BALB/cByJ, C3H/HeJ, DBA/2J, and CD-1 mice, but only in the A/J strain are reductions of DA observed. Finally, in the midbrain tegmentum, stressor exposure increased DA utilization in BALB/cByJ, CD-1, and C3H/HeJ mice, and in the former two strains, marked reductions of DA concentrations were evident, as well (Shanks et al., 1988; Tassin et al., 1980).

The behavioral effects of stressors are certainly complex, likely being subserved by the emergent properties of several transmitters. As such, it would be too simplistic to assume a one-to-one correspondence between a neurochemical and a behavioral change provoked by stressors. Nevertheless, the finding that stressors differentially influence vulnerability to central neurochemical alterations as a function of brain region, as well as the strain of animal employed, emphasizes the necessity of a multifactorial approach in the assessment of stressor effects. Apparently, in developing an animal model of depression, it may not be sufficient to consider any one strain (or species), behavior, or neurochemical system.

7. Summary

In accordance with the supposition that the effects of stressors in animals may be used as an analog of human depression, it has been demonstrated that:

1. Uncontrollable aversive events engender an array of behavioral changes, some of which are reminiscent of the symptoms that characterize depression in humans
2. Some of the neurochemical changes elicited by stressors, as well as receptor alterations, have also been implicated in human affective disorders
3. Treatments effective in alleviating depression in humans are also effective in eliminating some of the stressor-induced behavioral disturbances in animals.

In drawing the parallel between stress models in animals and the human syndrome, several caveats should be considered. Specifically, as in the case of humans where the symptoms of the illness may vary considerably across individuals, it seems that in animals pronounced strain differences exist with respect to the nature of the behavioral differences observed. Although differences in vulnerability exist with respect to the impact of stressors, these variations are expressed in terms of specific behaviors (or symptoms). Likewise, the effectiveness of antidepressant therapy in ameliorating stressor-induced behavioral disturbances is both strain- and symptom-specific.

It has been our contention that environmental insults engender numerous central neurochemical changes that may be essential for the organism to deal with ongoing or impending stressors. However, when these neurochemical systems are taxed, or when the course of a neurochemical adaptation in response to a chronic stressor does not progress readily, vulnerability to behavioral pathology is increased. The specific symptoms of the disorder are thought to be dependent on the precise neurochemical changes exerted by the stressor, as well as the brain region in which these occur. Likewise, aversive events may engender immunological changes that increase vulnerability to immunologically related disorders. Indeed, it seems that some of the conditions that lend themselves to immunological disturbances are similar to those that are associated with central transmitter alterations. To be sure, not all of the manipulations that affect one system affect the other, nor is it assumed that a one-to-one correspondence exists between central transmitter activity and immune functioning. Nevertheless, there are data that make it tempting to speculate that CNS alterations influence immune functioning, and conversely, immunological changes may affect CNS activity. As a working hypothesis, we have proposed that exposure to pathogens, by virtue of their central consequences, may come to influence affective state, much in the same way that physical or psychological stressors do. In effect, in considering the potential impact of stressors on the affective state, it may be appropriate to include pathogenic stimuli as one component of the stressor mosaic.

Although it may be of obvious value to identify a relationship between stressor-provoked neurochemical alterations and the specific behavioral changes that are encountered, such a pattern is not immediately apparent. We have argued previously

(Anisman and Zacharko, 1989) that stressors may induce cascading neurochemical changes that might subserve the primary and secondary symptoms of depression. It follows that the emergence of these symptoms will be dependent on, among other things, the sequencing of the neurochemical variations, as well as the central sites in which these occur. It is thus reasonable to suppose that the depressive symptoms will be a reflection of progressive neurochemical events, rather than those existant at any single point in time. Clearly, in order to determine the stressor-neurochemistry-depression topography, it is essential not only to consider a wide array of behaviors and the strain differences that exist in response to stressors, but also the dynamic neurochemical changes underlying the disorder.

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The Olfactory Bulbectomized Rat as a Model of Major Depressive Disorder

J. Steven Richardson

1. Introduction

1.1. Essential Characteristics of Major Depression and of Animal Models

The creation or discovery of animal models of psychiatric conditions, such as major depressive disorder, is fraught with many problems that are not encountered in the development of models in other areas of medicine. Some of these problems are the result of the apparent nature and complexity of the symptoms presented by the psychiatric patient. However, other problems reflect philosophical positions ranging from those based on the premise that primary depression does not fit the medical model and therefore is not a "disease" that can be modeled, to those holding that the emotional state of depression is so uniquely human that subhuman organisms cannot be used in its analysis. In evaluating animal models of any human disorder or condition, it is crucial for those using the models to keep abreast of developments and advances in the relevant clinical knowledge concerning the particular condition being studied. This is especially important for such disorders as essential hypertension or major depression where the central causative factors are not yet known, but where extensive clinical investigation is constantly expanding knowledge of the patients' condition. As more is learned about the clinical characteristics of the disorder, this information must be incorporated into the con-

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ceptual framework underlying the animal models of the disorder (Richardson, 1991).

Over the years, depression has been viewed as being the reaction to the loss of a valued person or object, the consequence of exposure to a level of stress that exceeds the person's ability to cope, or the expression of a genetically determined biochemical abnormality in the neural substrates responsible for mood, emotion, and memory (Richardson, 1984; Siever and Davis, 1985; Jesberger and Richardson, 1985a,1988; Richardson, 1990). Each of these etiologies is reflected in various animal models (Willner, 1984,1990; Jesberger and Richardson, 1985b; Richardson and Jesberger, 1986), and although investigations of the effects of loss or stress are important areas of research, current clinical studies of people with major depressive disorder indicate that loss and stress do not play causative roles in the development of major depression. Data from neuroendocrine, neurochemical, and neuropharmacological studies indicate that the neurobiology of people who develop major depression differs from that of people who do not. However, there are slight differences in the presenting symptoms of different major depression patients, and this suggests that, as currently used, the diagnostic label "major depression" represents a group of disorders, each based on slightly different neurochemical substrates that produce roughly similar symptoms (Richardson, 1989a). Refinements in the precision of diagnosis would be a great help for classifying homogeneous groups of patients, for identifying the specific pathological neurochemical substrates of a given patient, and/or for optimizing therapeutic interventions. However, it is now clear that neurochemical abnormalities, perhaps with reduced noradrenaline activity being common to all as originally suggested by Schildkraut and Kety (1967), underlie major depressive disorders. Consequently, any animal model of depression must reflect comparable neurochemical abnormalities, too.

The symptoms of major depression can be divided into three categories: mood, cognitive, and neurovegetative (Table 1). Although negative thoughts and feelings generally seem to be the major problem for the depressed person, verbal reports of these

Table 1
Symptoms of Major Depressive Disorder

- Mood
  - Sad, frustrated, hopeless
  - Irritable
  - Apathetic
- Cognitive
  - Inappropriate negative thoughts and ideas
  - Everybody will be better off when I am dead; I think I will kill myself
  - Everything has always been terrible, and it will never get any better
  - I am being punished; I am guilty, and I deserve it
  - I am worthless and everybody hates me; they always have and always will
  - Impaired concentration, confusion
- Neurovegetative
  - Loss of appetite, loss of interest in almost everything
  - Sleep disorder (REM advance and early morning awakening)
  - Neuroendocrine abnormalities
  - Loss of energy, lack of motivation
  - No libido
  - Cannot experience or anticipate pleasure

inner states are not helpful in differentiating the patient with major depression from the person with a very unpleasant life. Since antidepressant drugs do not lift the spirits or improve the mood of people whose limbic homeostatic mechanisms are already in balance, in deciding on the appropriateness of drug therapy, it is important to differentiate the person who appears depressed because of a recent divorce or job loss from the person who was divorced or fired because he or she had major depression and could not function in the marriage or the workplace. The former will not benefit from antidepressant drug treatment, whereas the latter will. The best clues for this differential diagnosis are provided by the behavioral neurovegetative symptoms and by a personal or family history of major depressive disorders. The neurovegetative abnormalities are the most reliable reflection of the disruption of limbic system function that appears to be responsible for major depressive disorder. Since it is the behavior of the patient that is most relevant in identifying major depressive disorder, the study of behavior in the animal models is both a necessary and a sufficient requirement. Any animal model of depression must have behavioral abnormalities that respond to antidepressant drugs in a manner comparable to the patient.

Another nearly universal characteristic of major depressive disorder patients is that it takes 2–3 wk of drug treatment with any of the currently available antidepressants before improvement is seen in the patient's symptoms. This indicates that the important therapeutic mechanism of antidepressant drugs is not the immediate direct action of the drug, but rather is the body's long-term reaction to the direct drug effect. Clinically effective antidepressant drugs have a variety of direct effects, yet they all show the 2–3 wk therapeutic time lag. Consequently, animal models of depression must be sensitive to a variety of different antidepressant drugs when given chronically, but not when given acutely.

In summary, an animal model of major depression must reflect the patient with major depression by having a neurochemical abnormality that results in behavioral abnormalities that can be normalized by clinically effective antidepressant drugs given chronically, but not by acute antidepressant drugs or by drugs lacking antidepressant properties in the patient. As has been discussed elsewhere (Cairncross et al., 1979; Leonard, 1984; Jesberger and Richardson, 1985b; Richardson and Jesberger, 1986; Richardson, 1989b,1991), the olfactory bulbectomized (OBX) rat best fits these requirements.

1.2. The Role of Olfaction in the Evolution of Emotions and Memory: A Mechanistic Hypothesis

The survival of any life form depends on the ability to detect and react appropriately to potential prey, predators, and, in the case of species blessed with sexual reproduction, mates. In the aquatic environment where life originated, the recognition of chemical signals represents a simple, straightforward, and very useful mechanism for interacting with membrane components of the future host as seen in viruses or bacteria, and for identifying the presence of food, mates, or enemies by monitoring chemicals drifting along in the ocean currents, as seen in coral polyps, clams, or fish. Chemical sensory transduction proved so useful that species kept this ability when they became land creatures, and the survival and reproduction of nearly all past and present members of the animal kingdom depend on the sense of smell. Not only was it necessary to detect chemical signals in the primordial seas, but evolutionary advantage was gained by reacting appropriately to these signals and by remembering what happened the last time a particular chemical stimulus was detected. Consequently, components of the chemical sensory transduction apparatus evolved into systems able to determine the appropriate response to a given chemical signal and to keep representations of the situation at hand to aid in producing the behavioral pattern with the greatest survival value. Over the course of evolution, organisms also developed the ability to detect various vibration signals, such as the very slow vibrations that activate the lateral line of fish, the shorter, faster vibrations that activate the auditory apparatus, or the very short vibrations that stimulate visual processes. These later evolving sensory capabilities also have very significant survival value, but by the time they appeared, the appropriateness and memory functions of the specializations of the chemical sensory system were well developed, and as a result, the functions of these structures were shared with hearing and vision. In mammals, the chemical sensory function is the sense of olfaction, and the appropriateness and memory functions are performed by structures evolving from the chemical transduction pathways, such as the amygdala, hippocampus, and other structures of the limbic system. As will be discussed below, the mammalian olfactory bulbs have extensive monosynaptic and polysynaptic contacts with limbic structures, and for a while in the early part of the twentieth century, the limbic system was called the rhinencephalon, or the nose brain, in recognition of the anatomical and presumed functional relationships among the olfactory sensory fibers, the olfactory bulbs, the olfactory tubercle, the entorhinal cortex, the amygdala, the hippocampus, and the septal area.

In many situations, the behavior with the greatest survival value is a behavior pattern that has to be maintained over a period of time in the absence of the survival-related event, or evoked in a situation in preparation for the appearance of the survival-related event. The appropriateness and representational neural systems also developed the ability to generate an emotional state and a memory trace that increased the probability that appropriate survival behaviors would be evoked in a given situation. This emotional-memory function modulated and channeled the basic survival reflex centers in the hypothalamus (the four Fs of hypothalamic function: feeding, fighting, fleeing, and reproductive behavior) as needed to maximize the survival value of the behavior elicited in any given situation. As such, the emotions evolved as reinforcers that function to increase the chance of survival of the organism and the species over and above the basic needs and drive states governed by the hypothalamus. The emotions are basic, hard-wired functions of the limbic system, and seem to act as positive or negative reinforcers (Table 2) to maintain behavior with high survival value. The feel good emotions are positive reinforcers and increase the frequency of behavior that results in the appearance of that emotion. The feel bad emotions are negative reinforcers, reducing the frequency of behavior giving rise to that emotion and increasing the frequency of behavior that avoids or reduces that emotion. Emotion attaches survival value onto situations, and increases the probability that the organism and the species will survive.

Emotions have a genetically determined biochemical basis, and like any other biological function, are subject to disruption and abnormal expression when there are abnormalities in the biological substrates. This results in pathological emotional states where the power of reinforcement contingencies is lost and the person is deprived of the survival value of the emotions (Table 2). In this sense, major depression and other mood disorders are not conditions of excessive emotion, but rather are conditions of a lack of emotion where the limbic structures have lost the abil-

Table 2
Hypothetical Framework for the Function of Emotion

Pleasant emotions[1]	Unpleasant emotions[1]	Pathological emotions[2]
Positive reinforcers	Negative reinforcers	Reinforcement system are inoperative[3]
Love Contentment Happiness	Anger Fear Sadness	Rage Anxiety Depression

[1] Emotion attaches survival value to incoming sensory data, directs memory storage, and reinforces behavior.

[2] Emotional states lacking survival value are pathological. Loss of contact with reality.

[3] If emotion = survival value, does no survival value = no emotion?

ity to evaluate appropriateness and to generate survival-maximizing emotions and behaviors.

2. The Olfactory Bulbs

In the rat, the olfactory bulbs make up about 4% of the total brain weight and have a five-layer laminate structure that compares in complexity to the three layers of the hippocampus and the six layers of the cortex. Based on evoked potential and fiber tracing studies (summarized by Cain, 1974; Jesberger and Richardson, 1988), cells in the olfactory bulbs project directly to the olfactory tubercle, the piriform (Cattarelli and Cohen, 1989) and prepiriform cortices, the entorhinal cortex, the amygdala, the hippocampus, and the supraoptic nucleus of the hypothalamus (Hatton and Yang, 1989). Polysynaptic projections are made to the lateral habenula, and to various thalamic and hypothalamic areas. The olfactory bulbs receive input from the locus ceruleus, raphe nuclei, diagonal band, piriform cortex, amygdala, and hippocampus. Intrinsic neurons in the bulbs synthesize dopamine, enkephalin, gamma aminobutyric acid (GABA), substance P, thyrotropin-releasing factor, glutamate, and aspartate. The external plexiform layer of the olfactory bulbs exhibits the highest density of benzodiazepine receptors of any brain area studied (Richards et al., 1988) and is among the brain areas showing the highest number of insulin receptors (Matsumoto and Rhoads, 1990). (*See* Hirsch [1980], Halasz and Shephard [1983], and Jesberger and Richardson [1988] for more detailed discussions of the neurochemistry of the olfactory bulbs.)

2.1. Procedure for Bilateral Olfactory Bulb Removal

Adult male rats, housed in group cages under standard conditions for at least 1 wk following delivery from the supplier, are anesthetized (e.g., pentobarbital 50 mg/kg ip), their heads are shaved, and each rat is placed in a Kopf stereotaxic apparatus with the nose bar set at 0. A midline longitudinal incision is made for about 1 cm on either side of bregma, and the skin and periosteum are retracted. Two 2-mm diameter holes with centers 6 mm anterior to bregma and 2 mm on either side of midline are drilled with care taken not to penetrate the dura with the drill bit. The area is cleaned of debris with swabs or absorbant applicators (wooden shaft Q-tips™), and the dura is perforated with a hyperdermic needle. For sham lesion rats, the holes are now plugged with Gelfoam® (Upjohn) and the wound closed. We have found 7.5-mm Michel wound clips (Justrite Wound Clips®, Clay-Adams) to be a quick and cheap way to close the incision. For rats destined for the bulbectomy group, the base of each bulb is severed with a bent microknife (Fig. 1; American Dental Mfg. Co., FC1090, 8A) avoiding damage to frontal cortical tissue. The bulbs are aspirated using a water faucet vacuum source and a probe with finger-tip vacuum control constructed from 2-mm OD stainless-steel tubing (Fig. 1). When all bulb tissue has been removed, the holes are plugged with Gelfoam® and the incision closed with wound clips. Following recovery from the anesthetic, the rats should be kept in single cages for 2 wk prior to any experimental manipulation. The accuracy of the bulbectomy procedure is assessed visually at the end of the experiment by removing the brain from the skull, and checking for the absence of residual bulb tissue and for the lack of damage to the cortex (Jesberger and Richardson, 1986a).

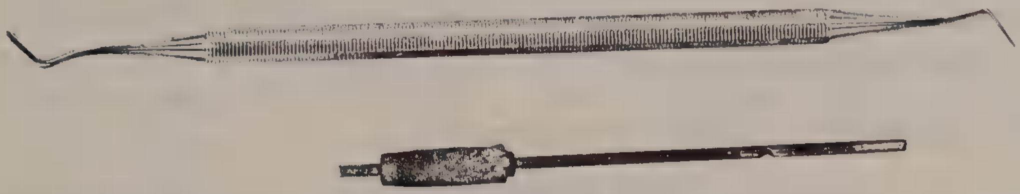

Fig. 1. (Top): Dental plastics tool used to sever olfactory bulb stalks. (Bottom): Stainless-steel tubing used to aspirate olfactory bulbs. Vacuum tubing is placed over enlargement on the left. Right end is inserted into hole drilled in skull, and force of suction is regulated by finger pressure sealing the hole (notch) cut near the right end.

A rapid method for removing the brain is as follows. Depending on local regulations, it may be necessary to induce light ether anesthesia in each rat prior to sacrifice. Decapitate the rat with a guillotine or, after ether anesthesia, with scissors cutting between cervical vertebrae, and use Mayo scissors to cut the scalp up to the eye sockets. Cut between the eye sockets with the scissors (or edge cutting wire cutters for rats over 350 g), cut muscle, and remove vertebrae if any from the foramen magnum. Insert one blade of the scissors into the foramen magnum parallel to the top of the skull, and cut to the side of the skull. Repeat on the other side, and pry the bone flap up breaking it off at the posterior edge of the skull. Insert one blade of the Mayo scissors between the brain and the skull with the cutting edge of the scissors next to the bone. Cut through the side of the skull and continue as far forward as possible. Repeat on the other side, and pry off the top of the skull. Remove the dura, especially the piece between the cortex and the cerebellum. Insert closed curved eye forceps down the side of the skull next to the bone in about the middle of the cortex. Turn the forceps so the tips ride along the bottom of the brain cavity, and lift the brain out. Pinch off the pituitary stalk and the facial nerves (the jaws or vibrissae will twitch), and the brain is ready for inspection and dissection.

For each new investigator, the investment of adequate time and rats in mastering the bulbectomy surgical procedure is very important before beginning any experiment. Any bulb tissue remaining connected to the brain, and any damage to the cortex, will alter the bulbectomy behavior syndrome and will necessitate discarding the data collected on that particular rat. Precise, consistent bulbectomy gives consistent, reproducible behavioral and biochemical data.

2.2. Bulbectomy Behavioral Testing

Although olfactory bulbectomized (OBX) rats demonstrate a wide range of behavioral abnormalities (Cain, 1974; Leonard and Tuite, 1981), the stepdown passive avoidance paradigm provides a reliable, easily assessed measure of OBX impairment (Thorne and Rowles, 1988; Tiong and Richardson, 1990) and the consequences of drug interventions (Jesberger and Richardson, 1986a). In this procedure, the rat is placed on an 11 × 11 cm wooden platform raised 4 cm above the electrifiable grid floor of a 30 × 30 × 20 cm Plexiglas™ chamber. If the rat steps off of the platform with all four feet, a 5 mA scrambled footshock is administered for 1 s. The rat is removed from the chamber for 30 s, and then replaced on the wooden platform. The footshock sequence is repeated until the rat remains on the platform for 1 min. Normal and sham rats reach the learning criterion after two to four trials. OBX rats require 10 to 20 trials. Nonparametric statistical analysis, the Mann-Whitney U-test, indicates that the OBX impairment of passive avoidance learning is invariably highly significant.

2.3. Neurochemical Changes Following Bulbectomy

As might be expected, when a brain area containing as many different neurotransmitters as the olfactory bulbs is removed, a wide range of neurochemical changes are observed following bulbectomy. The observation that initiated the evaluation of the OBX rat as a model of depression was the report by Pohorecky et al. (1969) of reduced noradrenaline levels in the telencephalon of OBX rats, a finding confirmed by Cairncross et al. (1975) and Edwards et al. (1977). As reviewed by Hirsch (1980) and Jesberger and Richardson (1988), OBX also reduces dopamine and serotonin levels in the telencephalon, serotonin and acetylcholine levels in the amygdala, and glutamate and aspartate levels in the cortex. After OBX, the amygdala also shows increased GABA levels (Jancsar and Leonard, 1983), increased estrogen receptors (McGinnis et al., 1985), increased α_2-adrenoceptors (Petterborg et al., 1989), increased affinity of the high-affinity β adrenoceptors (Tiong and Richardson, 1990), and reduced androgen receptor binding (Lumia et al., 1987). Other limbic system areas, such as the hippocampus (Jesberger and Richardson, 1986b; Tiong and Richardson, 1990), also show OBX effects on receptors, but the cortex does not (Tiong and Richardson, 1990). Although the brain areas responsible for specific OBX behavioral abnormalities remain to be identified, there is a very interesting parallel between altered amygdaloid function in the OBX rat and the inability of the OBX rat to learn passive avoidance tasks where the rat is required to alter predominant behavior patterns in light of environmental feedback on the appropriateness of the behavior. It was hypothesized nearly 20 years ago that the function of the amygdala was to evaluate behavior in terms of its environmental consequences and to alter behavioral output to make it appropriate for the given environmental situation (Richardson, 1971,1973). Obviously, an enormous amount of research remains to be done, but the hypothesis that the behavioral abnormalities of the OBX rat and of the major depression patient are the result in part of impaired amygdaloid function is currently supported by a great deal of circumstantial evidence.

2.4. Effects of Antidepressant Drugs on the Behavior of OBX Rats

One of the great attractions of the OBX rat as a model of depression is the remarkable similarity in drug effects between the OBX rat and the major depressive disorder patient. As discussed above, the depressed patient's personality and outlook on life return to the predepression state only after the antidepressant drug has been given for 2–3 wk. Chronic drug administration lifts the depression; acute drug exposure does not. Also, unlike the neurochemical homogeneity of the currently available neuroleptic drugs, all of which are dopamine receptor

Table 3
Drugs Correctly Identified by the OBX Model:
Drugs Giving a True Positive Antidepressant Response

Classic antidepressants	Amitriptyline
	Imipramine
	Desipramine
	Doxepin
	ECT
Selective serotonin uptake blockers	Fluoxetine
	Viloxazine
	Zimelidine
	Sertaline
	ORG 6582
Novel antidepressants	Mianserin
	ORG 3770
	Iprindole
	Bupropion
	Nomifensine
	Alprazolam
	Adinazolam
	Progabide (GABA agonist)

blockers, the pharmacological effects of antidepressant drugs cover a wide range of neurochemical mechanisms. Yet no matter what the direct effect of any particular antidepressant drug, clinical improvement is seen in the patient only with chronic treatment, and so too with the behavioral abnormalities of the OBX rat. Bulbectomy-induced behavioral deficits are normalized only by the chronic administration of drugs with antidepressant effects in patients with major depressive disorder (Table 3). The acute administration of antidepressants is not effective, nor is the administration of drugs belonging to other therapeutic classes (Table 4). To date, the OBX rat has made only two errors in drug screening (Table 5). The antispasmodic agent, baclofen, a $GABA_B$ agonist, improves OBX behavioral deficits, whereas the mono-

Table 4
Drugs Correctly Identified by the OBX Model:
Drugs Giving a True Negative Antidepressant Response

Amphetamine
Chlorpromazine
Haloperidol
Lithium
Diazepam
Chlordiazepoxide
Phenobarbital
Reserpine
Clonidine

Table 5
Drugs Not Correctly Identified by the OBX Model

False positive	False negative
Baclofen (a $GABA_B$ agonist)	Tranylcypromine (and other MAOIs)

amine oxidase inhibitor antidepressant drug tranylcypromine does not. The neurochemical effects of baclofen in OBX rats have not yet been studied extensively, but baclofen does seem to have some action on limbic emotional centers. Although there are no clinical reports of an antidepressant action of baclofen, GABA has been implicated in depression (Lloyd and Pilc, 1984), and there are numerous reports of inducing depression in patients by the abrupt discontinuation of high-dose baclofen therapy (Keegan et al., 1983). Since the syndrome seen during drug withdrawal is the opposite of the drug effects, baclofen could have some unrecognized antidepressant action in patients, too. The lack of effect of tranylcypromine and other monoamine oxidase inhibitors (MAOI) is more problematic for a model of depression, but this may be related to the specific neurochemical effects of OBX and the heterogeneous neurochemical nature of patients labeled as "depressed." The MAOI drugs are not very effective with typical major depression patients with the symptoms presented in Table 1, but the MAOIs are of marked benefit in panic attack and in atypical depression where the symptoms

Table 6
Parallels Between OBX Rats and Major Depressive Disorder Patients

Olfactory bulbectomized rat	Major depression patient
Neurochemical substrate demonstrated to include abnormalities in noradrenaline, serotonin, GABA, acetylcholine, and other neurotransmitters	Neurochemical substrate appears to include imbalance in noradrenaline, serotonin, GABA, acetylcholine, and other neurotransmitters
Neuroanatomical substrate demonstrated to include dysregulation of limbic-hypothalamic axis	Neuroanatomical substrate appears to reflect dysregulation of homeostatic neural activity in limbic-hypothalamic axis
Characteristic response specific to chronic but not acute antidepressant drugs except for those in the monoamine oxidase inhibitor class	Characteristic response specific to chronic but not acute antidepressant drugs; monoamine oxidase inhibitors effective in atypical depression subgroup
Elevated plasma adrenal steroids (corticosterone)	Elevated plasma adrenal steroids (cortisol)

(continued)

include overeating and oversleeping. These clinical observations suggest that the OBX rat is a good model of typical depression, but not of related mood disorders.

2.5. Parallels Between OBX Rats and Major Depressive Disorder Patients

Although the OBX rat obviously cannot verbalize guilt, hopelessness, and suicidal thoughts, this is not a serious problem in using OBX rats to study depression (Richardson, 1989b). There are numerous hints from behavioral studies of OBX rats that suggest parallel or potentially related characteristics shared by the OBX rat and the depressed patient. These are summarized in Table 6. Although there are no reports yet of lesions or neural degeneration in the brains of patients with major depressive disorder, there is a large amount of literature on depression in patients that have suffered neuronal death. Neuronal loss in

Table 6 *(continued)*

Olfactory bulbectomized rat	Major depression patient
Learning deficits in task involving positive or negative reinforcement	Insensitive to positive and negative reinforcement
Abnormal daily eating pattern; reduced weight gain	No interest in food; weight loss
Abnormal lordosis and other sexual behaviors	No interest in sex
Disruption in nest building and food gathering	No interest in the future
Not testable	No interest in work, hobbies, TV, and so forth
Abnormal sleep pattern	Sleep disorder
Hyperactive	Agitated
Hyperreactive, increased aggression and muricide	Hyperirritable
Passive avoidance deficit	Suicidal
Not testable directly, but performs perseverative maladaptive behavior	Cognitive disorder; verbalizes persistent inappropriate ideas
Not testable	Depressed mood, sad, hopeless; verbalizes guilt, frustration, and misery

the limbic structures is very dramatic in Alzheimer's Disease (Jones and Richardson, 1990), and depression is frequently seen in the early stages of this disorder. For some people with dementia of the Alzheimer's type, depression may be the initial presenting symptom. The occurrence of depression is also very high in stroke patients, especially in those with ischemic neural destruction in the anterior parts of the brain. In fact, stroke patients are the only group, other than the families of major depression disorder patients, where the prevalence of depression, estimated to be about 50% (Finklestein et al., 1982), exceeds the population norm of 5–10%. The symptoms of depression in these stroke patients are related to the location of the cell loss, not to the severity of impairment and are very similar to the symptoms of major depressive disorder (Robinson et al., 1983). Moreover, the depressed stroke patients show mood improvement to chronic, but not acute treatment with antidepressant drugs (Lipsey et al., 1986), just like the patients with major depressive disorder and just like the behavioral improvement in OBX rats. Although the behavioral and pharmacological parallels among these three groups do not indicate that identical neural substrates are necessarily involved, data from the study of the patients and the model will advance the understanding of limbic function and will help identify the contributions of limbic structures to normal and pathological behavior.

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A Cholinergic Supersensitivity Model of Depression

David H. Overstreet and David S. Janowsky

1. Introduction

It is now widely accepted that depressive disorders in humans are heterogeneous. Therefore, it is very likely that several distinctly different animal models of depression can be useful. Because of the heterogeneity of the manifestations of depressive disorders, it is important for developers of animal models to indicate which specific aspects of the illnesses their models mimic. The present chapter will describe the development and essential characteristics of the Flinders Sensitive Line of rats, selectively bred for increased cholinergic function. To provide a background for this discussion, the phenomenology of human depression, with a focus on cholinergic mechanisms and animal models of depression, will be briefly outlined.

1.1. Phenomenology of Human Depression

Individuals suffering from depressive disorders may be labeled bipolar if they have suffered from both manic (hypomanic) and depressive episodes, or unipolar if they have suffered from only depressive episodes. Numerous behavioral and physiological changes occur during a depressive episode. Besides changes in mood, the most characteristic are changes in motor activity, appetite, sleep, and weight (e.g., Akiskal and McKinney, 1973). The "classical" depressive individual will exhibit reductions in each of these parameters; the "atypical" depressive individual may often exhibit increases. A decrease in rapid eye

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movement (REM) sleep latency and an increase in REM sleep density are also characteristic, although not exclusively so, of classical depression, as is activation of the hypothalamo-pituitary-adrenal axis (e.g., Carroll et al., 1981; Shiromani et al., 1987), with increased cortisol and adrenocorticotropic hormone (ACTH) secretion and resistance to glucocorticoid suppression.

A number of reports have suggested that the neuroendocrine, mood, and sleep abnormalities mentioned above can be related to increased central cholinergic sensitivity. For example, it has been well established that the concentrations of cortisol and ACTH are elevated following administration of cholinergic agonists (Davis and Davis, 1980; Janowsky and Risch, 1984; Risch et al., 1983). Similarly, REM sleep latency and density are, respectively, reduced and increased by cholinergic agonists (Shiromani et al., 1987). It is also widely accepted that the behavioral changes commonly seen in classical depression are mimicked in normal individuals and exaggerated in depressive individuals following the administration of cholinergic agonists (e.g., Dilsaver, 1986; Janowsky et al., 1972,1974,1980). Thus, cholinergic agonists and cholinesterase inhibitors, in parallel with the characteristics of depression, cause psychomotor retardation, decreases in thoughts and thinking, withdrawal, and low mood, as well as ACTH and cortisol release and sleep changes characteristic of depression.

The cholinergic supersensitivity model of depression has received extensive support from pharmacological "challenge" studies. Here, depressive and normal individuals are given cholinergic agonists or cholinesterase inhibitors, and behavioral and/or physiological variables are measured. During an active episode of depression, individuals have been reported to be more sensitive to the behavioral (Janowsky et al., 1972,1980,1985; Nurnberger et al., 1983; Risch et al., 1981a,b), REM sleep-inducing (Jones et al., 1985; Sitaram et al., 1980,1982,1987), and neuroendocrine (Akiskal and McKinney, 1973; Risch et al., 1983) effects of the agonists. Furthermore, some studies of agonist-induced REM sleep suggest that the cholinergic supersensitivity may be a trait marker (Sitaram et al., 1987); others have found that the cholinergic supersensitivity disappears as the patient recovers, suggesting a state marker (Berger et al., 1989; Nurnberger et al., 1989). These differences could relate to the diagnostic heterogeneity referred to above, since the majority of the patients in the "trait" study were bipolar (Sitaram et al., 1987), and the majority of those in one "state" study were unipolar (Berger et al., 1989). Further experiments are necessary to resolve this issue.

Despite much evidence for cholinergic supersensitivity in depressive patients and for the concept that cholinergic agonists and cholinesterase inhibitors cause syndromes paralleling depression, there have been no parallel findings with respect to cholinergic receptors (mAChR). Studies of brain tissues from individuals committing suicide have focused on the cerebral cortex, a possibly inappropriate brain region for such studies *(see below)*, and have not reported any remarkable findings (Kaufman et al., 1984; Meyerson et al., 1982). There was an early report of increased mAChR binding on fibroblasts from mainly bipolar patients (Nadi et al., 1984), but numerous workers could not replicate this finding (e.g., Kelsoe et al., 1985). More work is necessary, because the reports to date are just not extensive enough to permit any satisfactory conclusion.

In summary, there is extensive evidence to indicate that humans with affective disorders are supersensitive to centrally acting cholinergic agonists. This supersensitivity correlates well with the typical behavioral and/or physiological changes reported to occur in depressive disorders. However, to date, this supersensitivity has not been related to basic changes in mAChR, but studies have been too limited anatomically to determine whether the changes do or do not exist. An animal model in which cholinergic supersensitivity exists could help to clarify these issues and to direct further research in humans.

1.2. Animal Models of Depression

There have been an extensive number of animal models developed to mimic various aspects of affective disorders (Katz, 1981; Maier, 1984; McKinney, 1984; Overstreet, 1989b; Porsolt, 1981; Willner, 1984; other chapters in this book). As indicated above, the existence of a large number of different animal models

els is consistent with the realization that depressive disorders may be heterogeneous. Nevertheless, the large majority of the animal models developed to date have neglected the genetic component, which has long been recognized to be involved in depressive disorders (e.g., Akiskal and McKinney, 1973; Gershon, 1982). Therefore, most models are "modeling" the environmental-stress component of depression, which has also been recognized to contribute to depressive disorders (Akiskal and McKinney, 1973). Potential genetic animal models of depression have received much less attention.

Because of the evidence presented above that cholinergic supersensitivity may be a trait/state marker for depressive disorders in humans, an animal model selectively bred to have increased cholinergic sensitivity could be useful. The Flinders Sensitive Line (FSL) and Flinders Resistant Line (FRL) of rats were selectively bred to differ in their sensitivity to the anticholinesterase diisopropyl fluorophosphate (DFP) (Overstreet et al., 1979; Russell et al., 1982). These lines also exhibit a number of differences in cholinergic function, as will be described below. Only these animals will be discussed in this chapter. Other possible genetic models of depression, such as the Roman High Avoidance (RHA) and Roman Low Avoidance (RLA) strains, are described elsewhere (Overstreet, 1991; Overstreet et al., 1988).

2. Cholinergic Supersensitivity

2.1. Selective Breeding of FSL and FRL Rats

The original intention of the selective breeding study was to establish an anticholinesterase-resistant line of rat for comparison with anticholinesterase-tolerant rats developed by chronic treatment; we were interested in comparing the mechanisms underlying resistance and tolerance (*see* Russell and Overstreet, 1987 for a fuller description). But, as so often happens in scientific investigations, the original intention of developing an anticholinesterase-resistant line was not fulfilled. Instead, we obtained a line of rats much more sensitive to DFP (Overstreet et al., 1979; Russell et al., 1982). In the majority of tests conducted to date, the so-called resistant line (FRL) has not been different from control Sprague-Dawley rats (e.g., Overstreet et al., 1984, 1986b).

The above pharmacological selection experiment differed in some important ways from prior studies. Both sexes were tested, and selection pressure was placed on both sexes. In the initial stages, the rats were exposed to a 17-d adaptation period during which they learned to drink from especially calibrated drinking tubes in half-hour sessions. Water intake, core body temperature, and body wt were the three dependent variables studied. At the end of the 17-d adaptation period, a 1 mg/kg dose of DFP was administered and the decreases in temperature (at 4 h), water intake (at 24 h), and body wt (24 h) were recorded. Rats within their respective line and sex category were rank-ordered for each variable, and the average ranks were determined. Based on average ranks, the six most-affected (sensitive) males and females were then mated, as were the six least-affected (resistant) males and females. Progeny from subsequent generations began a similar adaptation period at 70 d of age, and only the six most-resistant males and females from the FRL rats and the 6 most-sensitive males and females from the FSL rats were kept for subsequent breeding.

There were obvious differences between the lines within eight generations (Overstreet et al., 1979). However, regression analyses of the generation/effect curves failed to reveal any significant change in temperature, water intake, or body wt in the FRL rats, indicating no change in response with generations (*see* Fig. 1). On the other hand, there was a significant downward trend for these measures in the FSL rats, suggesting that they were becoming more sensitive to DFP in subsequent generations (*see* Fig. 1). The increased sensitivity of FSL rats to DFP was confirmed in a subsequent study and was shown to generalize to other measures, such as open-field activity and operant responding for water reward (Russell et al., 1982).

Initial studies, designed to determine the mechanisms underlying the increased sensitivity of FSL rats to DFP, focused on enzymes known to be inhibited by DFP. Since insects genetically resistant (insensitive) to anticholinesterases have insensitive acetylcholinesterase (AChE) enzymes (*see* Russell and

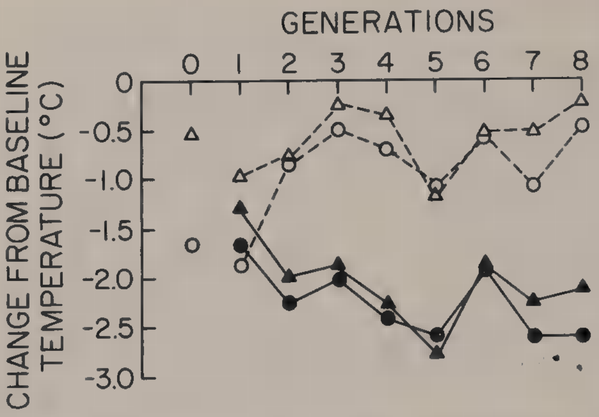

Fig. 1. Change in hypothermic responses to DFP in FSL and FRL rats during the first eight generations of genetic selection. The data represent the means for 15–22 rats/group. ▲—▲ = FSL females; △—△ = FRL females; ●—● = FSL males; ○—○ = FRL males. Adapted from Overstreet et al. (1979).

Overstreet, 1987), it was reasonable to hypothesize that FSL rats might have a more sensitive AChE enzyme. However, both in vitro and in vivo studies indicated that the AChEs from the FSL and FRL strains had equal sensitivities to inhibition by DFP (Overstreet et al., 1979; Sihotang and Overstreet, 1983). In addition, two serum enzymes inhibited by DFP, butyrylcholinesterase and carboxylesterase, are present in equal amounts in the two lines (Overstreet et al., 1979,1990a). Therefore, the increased sensitivity of the FSL rats to DFP cannot be accounted for by any changes in enzymes with which DFP interacts.

2.2. Cholinergic Challenges

As described above, the pharmacological-challenge approach has been widely used in human studies in recent years to compare the sensitivity of people with some condition or disease with a normal population (e.g., Sunderland et al., 1988). Such a strategy has been widely used by animal researchers, including ourselves, for many years. Because of our experience with cholinergic challenges in DFP-tolerant rats (e.g., Overstreet et al., 1973,1974; Russell et al., 1971), we were in a good position to look at the FRL and FSL rats. The initial observation was quite illuminating: the FSL rats were more sensitive to the behavioral effects of muscarinic agonists and less sensitive to the behavioral effects of muscarinic antagonists (Overstreet and

Russell, 1982) than FRL or randomly bred rats. Thus, they were opposite in sensitivity to DFP-tolerant rats, which are less sensitive to muscarinic agonists and more sensitive to antagonists (Overstreet et al., 1974; Russell et al., 1971) than randomly bred rats. Since DFP-tolerant rats were known to have reduced numbers of muscarinic acetylcholine receptors (mAChR) (e.g., Schiller, 1979), it was predicted that FSL rats would have increased numbers of mAChRs. As we will see below, this prediction was partially correct.

Subsequent studies, using a range of behavioral and physiological indices, have confirmed the increased sensitivity of the FSL rats to muscarinic agonists (Crocker and Overstreet, 1984; Netherton and Overstreet, 1983; Overstreet et al., 1986a,b; Pepe et al., 1988; Wallis et al., 1988). Most recently, the sensitivity of FSL and FRL rats to a number of selective muscarinic agonists and antagonists was examined. The FSL rats exhibited supersensitive behavioral and/or physiological responses to McN-A-344, an m_1 agonist; oxotremorine-M, an m_2 agonist; pirenzepine, an m_1 antagonist; and AFDX-116, an m_2 antagonist; but were subsensitive to the nonselective antagonist, scopolamine (Schiller et al., 1988) Although more work is required to understand the response of FSL rats to antagonists, it is abundantly clear that FSL rats exhibit cholinergic supersensitivity parallel to that seen in depressed humans (Janowsky et al., 1980; Risch et al., 1981a,b, 1983).

In fact, the differential sensitivity of FSL and FRL rats to muscarinic agonists is so reliable that it is used to select animals for further experiments. It has been established that the rats can be differentiated as early as 30 d of age, soon after weaning and well before adulthood (Overstreet, unpublished observations, 1987). The typical screening involves injecting a standard dose of the noncentrally acting antagonist, methyl atropine (2 mg/kg), to block the peripheral effects of the agonist, approx 15 min before a standard dose of the centrally acting agonist, oxotremorine (0.2 mg/kg). Core body temperature is recorded via a thermistor probe 30 min after the injection of oxotremorine. A typical FSL male rat will exhibit a drop in temperature of approx 3.0°C, whereas the drop for a typical FRL male rat will be only

1.6°C. Corresponding drops in temperature for female counterparts are usually 0.5°C lower (Netherton and Overstreet, 1983). This procedure has been successfully used for at least five years with the original colony at Flinders University in Australia and, more recently, with colonies that have been established in the Department of Psychiatry at UC San Diego in 1988 and in the Center for Alcohol Studies at the University of North Carolina in 1989.

Recent studies indicate that the muscarinic sensitivity in the FSL rats is influenced by both additive and dominance genetic variance. Cross mating studies were carried out between the FRL and FSL rats to obtain F_1 and F_2 progeny and F_1 backcrosses to each line. Then operant responding for water reward, open-field activity, and core body temperature were recorded after administration of the muscarinic agonists, oxotremorine and arecoline. Computer-assisted analysis of the data indicated that genetic variance was exclusively in consequence of additive and dominance factors. Since the F_1 and F_2 progeny more closely resembled the FSL parents, it appears that muscarinic sensitivity may be influenced by dominant genes (Overstreet et al., submitted). This finding may have some relevance to recent studies in bipolar depression, which have implicated dominant modes of inheritance (e.g., Egeland, 1988).

2.3. Muscarinic Receptor Binding

Our first report of mAChR binding in the FSL and FRL rats indicated that there was a regional specificity for the changes: The FSL rats exhibited increases in mAChR binding in the striatum and hippocampus, but not in the cerebral cortex (Overstreet et al., 1984). Parallel studies of endogenous and deuterium-labeled ACh concentrations suggested an increase in synthesis in the cerebral cortex of FSL rats compared to FRL or randomly bred rats, but there were no differences in the striatum, hippocampus, or pons/midbrain (Overstreet et al., 1984). Thus, there appears to be a presynaptic cholinergic overactivity in the cerebral cortex, and a postsynaptic cholinergic supersensitivity in the striatum and hippocampus. The increased concentrations of mAChRs in the striatum and hippocampus and absence of an increase in the cerebral cortex have recently been replicated (Pepe et al., 1988). In preliminary studies, both m_1 and m_2 muscarinic receptor subtypes were found to be elevated in the striatum and hippocampus, and unchanged in the cerebral cortex of FSL rats (Schiller et al., 1989). These preliminary studies have also demonstrated that the FSL rats have higher numbers of m_2 binding sites in the hypothalamus, the site of temperature regulation, and in midbrain/pons, the site of generation of REM sleep (Shiromani et al., 1987).

The regional specificity of the mAChR elevations may be relevant to the lack of central mAChR differences between suicide victims and normal individuals (Kaufman et al., 1984); i.e., the cerebral cortex may have been an inappropriate region to study. As indicated previously, data on mAChR concentrations in limbic and other regions likely to participate in the behavioral and physiological dysfunctions observed in depressed individuals would be useful.

2.4. Measurement of Second Messengers

Although there are significant elevations of mAChRs in the FSL and FRL rats, these are only modest, on the order of 20% (Pepe et al., 1988). Also, the differences have been routinely observed only in male rats, those between females being smaller or nonexistent (Overstreet et al., 1989). Consequently, it is possible that receptor elevation is not the sole mechanism underlying the cholinergic supersensitivity in the FSL rats. It is well known, for example, that chronic treatment with antidepressants may induce both downregulation of β-adrenergic receptors and uncoupling of receptors to second messengers (Wachtel, 1989). The FSL rats may have a more efficient coupling of mAChR with second messengers, phosphatidylinositol (PI), and cyclic adenosine monophosphate (cAMP). As yet, no studies exploring this possibility in FSL and FRL rats have been completed, but preliminary studies are underway.

3. Behavioral Features of FSL Rats

Because the cholinergic system is involved in virtually all behavioral and physiological functions, the established differences in central cholinergic function between the lines (e.g., Overstreet et al., 1984) may result in some behavioral and physiological differences. Indeed, in behavioral and physiological variables, a number of baseline differences between the FSL and FRL rats have been observed (Netherton and Overstreet, 1983; Overstreet et al., 1979; Russell et al., 1982). In this section, these differences will be described and related to what appear to be parallel changes in depressive disorders in humans.

3.1. General Activity

In the earlier selection studies (Overstreet et al., 1979; Russell et al., 1982), FSL rats were observed to have decreased baseline body wt and decreased open-field activity. Parallels to both of these changes are well documented to occur in humans who become depressed (at least in typical depression), but less information is known about these variables in euthymic individuals with a history of affective disorders. However, Wolff et al. (1985) have demonstrated that euthymic patients do indeed show a reduced activity compared to control subjects. This reduction was most apparent during the daylight hours, the active portion of the circadian cycle, which parallels our finding that the FSL rats were less active than the FRL rats during the dark, active portion of their circadian cycle (Overstreet, unpublished observations, 1985).

A study of sleep parameters in FSL and FRL rats under baseline conditions has established that FSL rats exhibit REM sleep abnormalities similar to those seen in depressive human (e.g., Sitaram et al., 1982; Shiromani et al., 1987,1988). These abnormalities include an increase in REM density and a more frequent REM-to-REM cycle, functionally equivalent to a shortened REM latency. There is good reason to believe that these abnormalities are related to the cholinergic supersensitivity observed in the depressed humans and the FSL rats (Shiromani et al., 1987).

The reduced latency to the onset of REM sleep (more frequent REM-to-REM cycle) observed in FSL rats is suggestive of abnormalities in other biological rhythms found in depressive individuals. It has been commonly suggested that circadian and biological rhythm abnormalities are associated with depression in humans (e.g., Wehr and Wirz-Justice, 1982; Wehr et al., 1983).

In particular, it has been proposed that a phase advance of biological rhythms predisposes toward depression (Wehr and Wirz-Justice, 1982). The phase advance of REM sleep described above in the FSL rats is thus consistent with the proposal that they represent an animal model of depression. Preliminary studies exploring this relationship have shown that the FSL rats have a phase advance of their body temperature rhythms compared to the FRL rats (Shiromani et al., 1991). These findings are very encouraging with respect to the hypothesis that FSL rats are good models of depression, and suggest that the biological rhythm and cholinergic supersensitivity models of depressive disorders can be integrated.

3.2. Avoidance Learning

Avoidance tasks are commonly conducted in two-compartment chambers and require the animal either to move actively from one side of the chamber to the other (active avoidance) or to remain in one compartment (passive avoidance) to avoid shock. On these tasks, the FSL and FRL rats, respectively, exhibit differences comparable to those observed for the Roman and Syracuse Low Avoidance rats and the Roman and Syracuse High Avoidance rats, which were selectively bred to differ in the learning of an active-avoidance response (e.g., Brush et al., 1979; Driscoll and Battig, 1982). The FSL and the low-avoidance rats perform poorly on an active-avoidance task (Brush et al., 1979; Driscoll and Battig, 1982; Overstreet and Measday, 1985; Overstreet et al., 1990c). In contrast, the FSL rats have significantly better memory on a passive-avoidance task (Overstreet, 1986) and the low-avoidance rats have also been reported to perform better on similar tasks (Driscoll and Battig, 1982; Iso et al., 1988). The poor performance of the FSL rats in the active-avoidance task is consistent with the observed cholinergic supersensitivity reported above.

These avoidance data, although internally consistent in the animal studies, do not appear to parallel the data from depressed humans. If only the data on active-avoidance are considered, then a good parallel exists, with the FSL rats being impaired, and most studies suggesting that memory processes, when altered, are impaired in depressed humans (McAllister, 1981). However, when the performance of the rats on other tasks is considered, the FSL rats appeared to have improved memories, and this result is at variance with human studies. An important consideration is the recognition that the relationship between cholinergic stimulation and memory processes in animals and humans is not monotonic. Improvement in memory is generally found only after intermediate concentrations of cholinergic agonists (Overstreet, 1984, 1989a), both higher and lower doses being ineffective or deleterious. Such data suggest that memory processes may be impaired in individuals whose cholinergic system is overstimulated, as might well be the case in depressive persons. However, it is important to note that memory for negative events has been reported increased in depressive individuals (Bower, 1981,1987; Breslow et al., 1981).

Recently we compared the FSL and FRL rats' abilities to perform in the radial-arm maze. If the increased mAChR concentrations in the striatum and hippocampus in the FSL rats are functionally significant, it would be expected that they would perform differently from the FRL. There was a nonsignificant trend for the FSL male rats to learn the maze more poorly (Overstreet et al., unpublished observations, 1988). It would be particularly valuable to conduct studies on euthymic, previously depressed patients with a history of depression to assess their memory processes; the prediction is that they would have better memories because of an increased cholinergic sensitivity that is not so high as to be pathological.

3.3. Operant Responding

In earlier studies (Overstreet and Russell, 1982; Russell et al., 1982), designed primarily to extend the variables for DFP sensitivity and to study the effects of muscarinic agonists and antagonists, it was found that there were differences between the FSL and FRL rats in terms of acquisition rates of learning an operant response for water reward. Since FSL rats took longer to acquire the response and responded at much lower (50%) rates on the final fixed-ratio (five bar presses per reward) task, these data may simply be a reflection of the locomotor activity differences reported earlier. Alternatively, since an overactive cholinergic system would be predicted to reduce the value of reward (e.g., Domino and Olds, 1968; Newman, 1972), the diminished operant responding observed in the FSL rats may be accounted for by the hypothesis that FSL rats obtain less reward from water. More extensive and sophisticated operant-responding studies are required to differentiate between the two suggestions made above. As yet no such studies have been conducted, but they could be very interesting, since there is considerable debate about which neurotransmitter(s) mediate the well-known lack of ability to experience pleasure (anhedonia) associated with depression in humans (German and Bowden, 1974; Sachar, 1985; Whybrow et al., 1984; Wise, 1978). Furthermore, it may be significant that many subjects receiving the cholinesterase inhibitor, physostigmine, report a state of apathy, feeling drained and fatigued, and a lack of interest that is also characteristic of retarded depression (Janowsky et al., 1974).

3.4. Stress-Induced Immobility

Although the relation between stress and depression is complex, many investigators feel that stress can be both a predisposing and a precipitating factor in causing affective disorders (*see* Anisman and Zacharko, 1982). Overstreet et al. (1988) recently suggested how cholinergic supersensitivity might be the mechanism underlying both of the above-mentioned influences of stress on depression (*see also* Janowsky and Risch, 1984). They proposed that animals chronically exposed to stressors might develop cholinergic supersensitivity through hormonal mechanisms (*see* Dilsaver, 1988), whereas genetic mechanisms underlie the cholinergic supersensitivity exhibited by the FSL rats. Because the FSL rats have elevated mAChRs (e.g., Overstreet et al., 1984), they are predisposed to become "depressed" when exposed to stressors, which promote the release of ACh (*see* Janowsky and Risch, 1984). In fact, our studies indicate that the FSL rats do exhibit a greater degree of immobility than do FRL rats when exposed to mild stressors (Overstreet, 1986; Overstreet et al., 1986a,1989). Compared to FRL rats, the FSL animals were signi-ficantly less active in an open field after exposure to footshock (1 mA, 2 s) and had longer periods of immobility in the forced swim test, developed originally by Porsolt (1981). In their recent review of the forced swim test, Borsini and Meli (1988) concluded that it was useful in screening for antidepressants in rats, but not in mice. Thus, the greater immobility shown by the FSL rats in the forced swim test may be a reflection of "depression." There is a need to test the FSL and FRL responses to other stressors and for further work with humans before more extensive generalizations can be made.

It is pertinent to reiterate the parallel between FSL rats and depressed humans in regard to the hypothalamo-pituitary-adrenal axis, which participates in many responses to stress. The astute reader will recall that both FSL rats and depressed humans exhibit elevated levels of adrenal hormones following a challenge with cholinergic agonists (Overstreet, 1986a; Risch et al., 1981a,b). Since these hormones might further sensitize cholinergic mechanisms (*see* Overstreet et al., 1988), there could be a positive feedback system in operation. Studies of corticotropic releasing factor (CRF), the hypothalamic hormone that stimulates the release of ACTH from the anterior pituitary, have revealed abnormalities in depressive disorders (Nemeroff, 1988) consistent with CRF hyperactivation. Collaborative studies on CRF in the FSL and FRL rats with Dr. Nemeroff's group are currently underway, and early results indicate a pattern of CRF differences between the two lines that is consistent with the known effects of ACh on CRF function and the proposed role of ACh in depression.

4. Changes in Other Neurotransmitters

The cholinergic hypothesis of depression has been less generally accepted than the catecholamine and/or indoleamine hypotheses, at least judging from the volume of published literature. However, it should be pointed out that the original conception of what is now commonly referred to as the cholinergic hypothesis was a balance model: Janowsky et al. (1972) proposed that the cholinergic and adrenergic systems were in balance in the central nervous system, just as in the peripheral nervous system, and that depression might arise *either* from an excess of cholinergic function *or* from a deficiency of adrenergic function. Since the FSL rats have a well-documented excess of cholinergic function, it is interesting to determine if there are any alterations in other neurotransmitter systems. The present section presents a brief summary of what we know about other neurotransmitters to date.

4.1. Balance Among Neurotransmitters

It is widely accepted that both behavioral and physiological functions are influenced by interactions of multiple neurotransmitters and/or neuromodulators (Pradhan and Bose, 1978). The studies supporting this concept are too numerous to enumerate. Instead, a few selected examples will be given to illustrate the point.

Locomotor activity in animals is known to be influenced by a wide range of drugs interacting with a diversity of putative neurotransmitter systems. The concept of a cholinergic inhibitory system has been discussed in previous sections. In balance with this system are dopaminergic and noradrenergic excitatory systems and serotonergic inhibitory systems (Fibiger et al., 1970; Gerson and Baldessarini, 1980). Stimulation of the GABAergic system also leads to behavioral depression. So, on the basis of drugs given systemically, it is possible to suggest that the cholinergic, GABAergic, and serotonergic systems are behaviorally inhibitory and the dopaminergic and noradrenergic systems are behaviorally excitatory. However, the conclusion based on systemic injections of drugs may not hold up when central injections are carried out. One example is that turning and stereotyped behaviors normally associated with the stimulation of dopaminergic neurons can also be seen in animals after intranigral injections of GABA agonists (e.g., Kaakkola and Kaarianen, 1980).

A second example is a physiological function, the regulation of core body temperature. Once again, many different neurotransmitters have been implicated and many controversies have arisen. Despite the fact that numerous investigators have shown that cholinergic agonists, when injected either centrally or peripherally, decrease core body temperature, some reviewers maintain that ACh is involved in mechanisms of heat gain, rather than heat loss (Bligh, 1979; Myers, 1980). These models have been complicated by the recent pharmacological data indicating that different receptors for serotonin may mediate heat loss and heat gain, respectively (Gudelsky et al., 1986), and that dopamine-stimulated hypothermia is mediated only by D2 receptors (Faunt and Crocker, 1987). In any case, there is sufficient evidence indicating that stimulation of either the cholinergic, dopaminergic, serotonergic (S1 receptor), or GABAergic systems will lead to hypothermia in rats.

A final example of neurotransmitter balances and interactions is the literature indicating that chronic drug treatment may lead to adaptations in multiple neurotransmitter systems. Chronic treatment with haloperidol, a dopamine antagonist, produces a subsensitivity to muscarinic agonists without any changes in mAChR; the supersensitivity to dopamine agonists in haloperidol-treated rats is usually accompanied by increases in dopamine receptors (*see* Overstreet and Yamamura, 1979). Workers have also reported increases in dopamine and GABA receptors in rats that have been chronically treated with DFP (Sivam et al., 1983), but there have not been any behavioral challenges with appropriate agonists to see if these receptor alterations have any functional significance. These studies provide the background for a working hypothesis that FSL rats, which have genetically altered cholinergic function, will also exhibit changes in other neurotransmitters known to be in balance with the cholinergic system.

4.2. Dopamine

Dopamine was the first neurotransmitter studied in the FSL and FRL rats because of the well-known cholinergic–dopaminergic interaction in the striatum (e.g., Klemm, 1989). There have also been a large number of reports on the interaction of dopaminergic and cholinergic systems on the basis of systemically administered drugs (e.g., Klemm, 1983,1985; Sanberg, 1983). Generally, these reports indicate that both cholinergic agonists and dopamine antagonists can produce behavioral depression and catalepsy, whereas dopamine agonists and cholinergic antagonists produce increased general activity and/or stereotyped behavior. Thus, dopaminergic and cholinergic systems appear to be opposing systems. Support for this notion comes from other studies that have shown that administration of a cholinergic agonist can substantially block the behavioral activating effects of a dopamine agonist (Fibiger et al., 1970; Janowsky et al., 1973).

The above discussion leads to the prediction that the FSL rats, which exhibit cholinergic supersensitivity, will be subsensitive to the behavioral activating and/or stereotypy-inducing effects of dopamine agonists. Such a subsensitivity was indeed found in our initial studies with apomorphine, a nonspecific dopamine agonist that interacts with both D1 and D2 receptors (Crocker and Overstreet, 1984). More recently, the more specific D2 agonist, LY 171555, has been used with confirmation of the original finding (Crocker and Overstreet, 1991). In addition, these more recent studies have shown that the FSL rats are more sensitive to the cataleptic effects of haloperidol and raclopride, dopamine antagonists with varying selectivity for the D2 receptor. A small, but statistically significant decrease in striatal D2 receptor binding sites was also detected in the FSL rats (Crocker and Overstreet, 1984). Thus, there appear to be secondary alterations in the dopaminergic system in FSL rats, which were predictable on the basis of previous knowledge about cholinergic–dopaminergic interactions.

A particularly striking finding with these dopamine-agonist challenges in the FSL and FRL rats is that the FSL rats are actually supersensitive to the hypothermic effects of apomorphine and LY 171555 (Crocker and Overstreet, 1984,1991). Thus, in the same group of animals given the same dose of dopamine agonists, the FSL rats exhibit a supersensitive response for one parameter (temperature) and a subsensitive response for another (stereotypy). These findings reinforce the notion expressed earlier that the types of neurotransmitter interactions may vary with brain region and/or function. As described above, the dopaminergic and cholinergic systems are opposing systems for the control of locomotor activity and stereotypy, but are parallel systems for the control of body temperature.

4.3. Serotonin

There is considerable evidence for the interaction of serotonergic and cholinergic systems (e.g., Nilsson et al., 1988; Samanin et al., 1978). Often the literature is contradictory, but there is general agreement that stimulation of both systems with moderate doses of agonists leads to behavioral depression (Gerson and Baldessarini, 1980; Klemm, 1989). As indicated above, the S1 and S2 receptors for serotonin may mediate opposite thermic responses (Gudelsky et al., 1986). This discovery helps to account for the confusing literature on serotonergic mechanisms in temperature regulation, including the suggestion that serotonin is involved in both heat loss and heat gain pathways (Myers, 1980). At the time our studies were conducted, this selectivity of serotonin receptors was not well known. Consequently, the use of *m*-chlorophenylpiperazine (mCPP) as the agonist and cyproheptadine as the antagonist was, in retrospect, not the best choice.

In any case, significant differences in thermic responses were exhibited by the FSL and FRL rats following treatment with both mCPP and cyproheptadine. Paradoxically, both compounds produced hypothermic responses that were significantly greater in the FSL rats (Wallis et al., 1988). Reference to subtype selectivity helps account for this paradoxical effect: Cyproheptadine, although an antagonist, is more selective for the S2 receptor (Peroutka and Snyder, 1979); mCPP has approx equal affinity for both receptors. Thus, both compounds may directly, or indirectly, lead to the activation of S1 receptors. If these speculations are correct, the FSL rats exhibit S1 supersensitivity.

The FSL rats also exhibited supersensitivity to the behavioral effects of mCPP, the serotonin agonist; a significantly greater reduction in bar pressing for water reward and of line crossings and rears in an open field was noted (Wallis et al., 1988). Much less is known about the receptor subtype mediating the behavioral depressant effects of serotonin agonists, but it is clear that the FSL rats were generally more sensitive to the effects of mCPP than were the FRL rats. Biochemical studies have not yet been conducted, so it is not possible to say whether these apparent differences in receptor sensitivity correlate to elevation of serotonin binding sites and/or decreases in serotonin turnover. It is also conceivable that the apparent sensitivity changes are just that; they arise because of cholinergic differences and the nature of the interactions between the serotonergic and cholinergic systems.

4.4. Benzodiazepine/GABA

In Section 4.2., it was pointed out that dopamine and ACh have a well-documented interaction in the striatum. There is also a well-documented role for GABA in this brain region, particularly in regard to striatonigral pathways, which may modulate the dopaminergic nigrostriatal pathways (*see* Bartholini, 1980; Decsi and Nagy, 1988; Martin, 1984). There are also GABA interneurons whose axons remain within the striatum. Some models of extrapyramidal motor function suggest that the cholinergic interneurons receive an input from incoming dopamine neurons and project to the outgoing GABA neurons or the GABA interneurons (Martin, 1984). In any case, it is likely that changes in GABA function may occur in the FSL rats because of their overactive cholinergic transmission influencing postsynaptic GABA neurons.

Using the challenge approach commonly used with cholinergic agonists, we found that the FSL rats were more sensitive to the behavioral depressant effects of both diazepam, a benzodiazepine agonist, and muscimol, a GABA receptor agonist, using operant responding for a water reward and open-field activity as measures of effects (Pepe et al., 1988). The FSL rats were confirmed to have increased sensitivity to muscarinic agonists on the same measures, and their mAChR binding sites were confirmed to be elevated in the hippocampus and striatum, but not in the cerebral cortex. Measurements of benzodiazepine binding sites revealed that the FSL rats also exhibited significantly greater binding in the hippocampus and the striatum, but not in the cerebral cortex, correlating to the mAChR binding (Pepe et al., 1988). There is no information on GABA receptors or turnover in the FSL or FRL rats, so mechanisms underlying the increased benzodiazepine/GABA sensitivity of FSL rats to muscimol is unknown.

4.5. Noradrenaline (Norepinephrine)

Because of the well-documented interactions between the sympathetic and parasympathetic divisions of the autonomic nervous system, mediated by noradrenaline (NA) and ACh, respectively, interest in the interaction of central noradrenergic and cholinergic systems has a longer history of exploration than the interactions mentioned above. In fact, the balance model of Janowsky et al. (1972) precedes some other balance models by a few years and basically explores noradrenergic/cholinergic interactions. Interest in the involvement of noradrenergic systems in behavioral phenomena declined somewhat in some circles in the late 70s with the growth of interest in dopamine and the possibility that dopamine might mediate some of the functions formerly ascribed to noradrenaline (e.g., Creese and Iversen, 1975).

The possibility of differences in noradrenergic sensitivity in the FSL and FRL rats has received very limited attention to date. Neither the behavioral depressant, nor the hypothermic, effects of the β-adrenoceptor agonist, salbutamol, were different in the FSL and FRL (Overstreet, 1989). The hypothermic effects of clonidine, an α-adrenoceptor agonist, were also similar in the FSL and FRL rats (Overstreet et al., 1989c). However, in both cases only a single dose was used, so this area has not been systematically studied. To date, no differences in noradrenergic sensitivity have been detected in the FSL and FRL rats.

Thus, as illustrated in Table 1, there appear to be differences between the multiple neurotransmitter systems of FSL and FRL rats. For hypothermia, the FSL rats are supersensitive to dopamine, serotonin, GABA, and benzodiazepine agonists, as well as muscarinic agonists. However, there is very little information on differences in neurotransmitter turnover or receptors in FSL rats, so it is difficult to interpret the drug-challenge data. This difficulty aside, these data can also be integrated with current thinking about the involvement of these neurotransmitters in depressive disorders. For example, the increased sensitivity to muscimol, a GABA agonist, is consistent with the hypothesis that GABA deficiencies exist in depression. Similarly, the supersensitivity to mCPP would be consistent with the serotonergic-

Table 1
Altered Drug Sensitivity in FSL and FRL Rats

Agonist drug	Receptor[a]	Measure and direction	Line with greater effect
Arecoline	mACh	↓ Operant response	FSL
Arecoline	mACh	↑ Corticosterone	FSL
Oxotremorine	mACh	↓ Temperature	FSL
Pilocarpine	mACh	↓ General activity	FSL
McN-343	m_1ACh	↓ Operant response	FSL
Oxotremorine-M	m_2ACh	↓ Operant response	FSL
Apomorphine	DA	↓ Temperature	FSL
Apomorphine	DA	↑ Stereotypy	FRL
LY 171555	D2	↓ Temperature	FSL
LY 171555	D2	↑ Stereotypy	FRL
mCPP	5-HT_1	↓ General activity	FSL
mCPP	5-HT_1	↓ Operant response	FSL
mCPP	5-HT_1	↓ Temperature	FSL
Diazepam	BZD	↓ General activity	FSL
Diazepam	BZD	↓ Operant response	FSL
Muscimol	GABA	↓ General activity	FSL
Muscimol	GABA	↓ Operant response	FSL

[a] Abbreviations: mACh, muscarinic acetylcholine; 1 and 2, subtypes of muscarinic receptors; DA, dopamine; 2, D2 dopamine receptor; 5-HT_1, serotonin S_1 receptor; BZD, benzodiazepine; GABA, gamma aminobutyric acid (*see* Overstreet et al., 1988; Pepe et al., 1988; Schiller et al., 1988; Wallis et al., 1988).

deficiency model of depression. In contrast, there are no apparent changes in noradrenergic sensitivity in the FSL rats, which might be expected to occur on the basis of a noradrenergic-deficiency model. Thus, the findings to date with respect to other neurotransmitter systems are consistent with some currently accepted models of depressive disorders, but not all.

5. Responses to Antidepressants

In his critical review of over two dozen animal models of depression, Willner (1984) placed particular emphasis on the ability of well-known antidepressants to overcome behavioral abnormalities, which are probably analogs of depression. Thus, although the forced-swim test and olfactory bulbectomy lead to opposite changes in activity, both models have been particularly useful for screening antidepressants (Borsini and Meli, 1988; Porsolt, 1981; Willner, 1984; chapter by Richardson, this volume). Studies of the effects of antidepressants on the "depressive tendencies" of the FSL rats have not been as frequent as those in the models mentioned earlier.

5.1. Imipramine

The first study of the effects of an antidepressant on FSL behavior was conducted with imipramine, a standard tricyclic antidepressant that is widely prescribed. The task utilized was performance in a two-way shuttle box. To keep learning to a minimum, the animals were given only six trials on each session, which were held at weekly intervals. The primary dependent variable was the time (in seconds) required to complete the six trials. During the initial session, the FSL rats required significantly longer times to complete the six trials than the FRL rats. The FSL and FRL were then separated into two matched groups; one group received chronic imipramine (10 mg/kg, ip daily) and the other received chronic control vehicle. Active avoidance performance in the shuttle box was retested after 7 and 14 d of chronic treatment. On both days, the FSL group receiving chronic saline required significantly more time than any of the other three groups. Thus, chronic imipramine improved the performance of the FSL group but not the FRL group—a finding that is consistent with the suggestion that imipramine counteracted the "depressive" tendencies of the FSL rats (Overstreet and Measday, 1985).

5.2. Lithium

Lithium, the prophylactic drug of choice for manic-depressive illness, has been postulated to work via cholinergic mechanisms, but not all workers agree with this presumption (Casebolt and Jope, 1989; Dilsaver, 1984; Lerer, 1985). In our first examination of the effects of lithium on FSL behavior, the drug was put in the drinking water of the rats and saline solution was added to reduce the toxic effects of lithium, as had been reported by others (Hines, 1986; Thomsen et al., 1976). Under these conditions, chronic lithium treatment did not counteract the FSL rats' greater tendency for behavioral immobility in the footshock test, nor did it alter cholinergic sensitivity or mAChR concentrations (Overstreet and Double, 1987). However, a substantial number of lithium-treated rats (up to 30%) lost weight, suggesting that this mode of administration may produce toxic effects. A more recent study has administered lithium in the food, with potassium added to reduce lithium toxicity. The animals in this experiment lost <5% of their initial body wt. The FSL rats treated chronically with lithium exhibited a reduced hypothermic response to oxotremorine (Shiromani et al., 1990), suggesting that it may counteract cholinergic supersensitivity, as earlier workers have reported (Dilsaver, 1984; Dilsaver and Hariharan, 1988). This second study measured only core body temperature, so it did not include a measure of the FSL rats' "depressive tendencies," and no conclusion can be made. Further work on the effects of lithium in the FSL rats is currently underway.

5.3. Rolipram

Rolipram, a new generation antidepressant that inhibits cAMP phosphodiesterase (Wachtel, 1983; Wachtel et al., 1988), has been reported to be an effective antidepressant in recent clinical trials. It is considered to be potentially very useful because it has hardly any anticholinergic effects, unlike imipramine (Wachtel et al., 1988). Chronic treatment with rolipram was able to counteract the increased shock-induced suppression of activity in the FSL rats, but, surprisingly, further increased cholinergic sensitivity without altering receptor concentrations (Overstreet et al., 1989). In addition, the rolipram-treated rats exhibited weight gain and polydipsia, side effects that could make this drug unpalatable in humans. The published clinical reports did not mention either of these effects as a problem, but rolipram has apparently been withdrawn from the market in Europe (Dilsaver, personal communication).

5.4. Bright-Light Exposure

Exposure to bright light has been regarded as an effective treatment for seasonal affective disorder, i.e., winter depression

(Wehr and Rosenthal, 1989). Dilsaver and colleagues have recently reported that exposure of rats to bright light can reverse or overcome the cholinergic supersensitivity brought about by stressing the rats (Dilsaver, 1988, Dilsaver and Alessi, 1987). Therefore, it was considered possible that the cholinergic supersensitivity and "depressive tendencies" of the FSL rats might also be overcome by exposure to bright light. The study, in which rats were exposed to bright (7400 lux) light during the light portion of a 12/12 light/dark cycle in a cross-over design, revealed that the hypothermic effects of oxotremorine were blunted by the bright-light treatment (Overstreet et al., 1990b). However, the bright light treatment had no influence on the behavioral depressant effects of oxotremorine, nor on the amount of time spent immobile in the forced swim test (Overstreet et al., 1990b). Thus, although we replicated earlier findings that bright light does blunt a hypothalamically mediated cholinergic response, there was no evidence of an antidepressant effect of the treatment in the FSL rats. Bright light appears to have a selective effect on hypothalamic mechanisms, in which retinal pathways project, whereas the depressive tendencies of the FSL rats may be mediated in other brain areas, such as areas of the limbic system.

In summary, a wide range of different types of antidepressants have been studied with respect to typical FSL behavior, although the total number of antidepressants examined is relatively small, and a diversity of effects has been noted. Imipramine and rolipram appear to have anti-FSL behavioral effects, although lithium and exposure to bright light did not. Given that rolipram led to an increase in cholinergic sensitivity, and lithium and exposure to bright light led to a reduced cholinergic hypothalamic sensitivity, there appears to be no clear-cut relationship between the effects of antidepressants and the depression-like tendencies of the FSL rats, at least as far as the drugs tested are concerned. However, as indicated previously, these studies have not been systematic nor complete enough to make any firm conclusions. Nevertheless, it is worth emphasizing that the FSL rats do exhibit an antidepressant response following chronic treatment with some antidepressants. Perhaps further studies with a greater number of antidepressants will clarify this situation.

Table 2
Similarities Between FSL Rats and Depressed Humans

FSL rats	Characteristic	Depressed humans
++[a]	Reduced body weight	+++
++	Reduced activity	+++
+++	Increased REM sleep	+++
+++	Enhanced responses to cholinergic agonists	+++
++	Elevated mAChRs	+
++	Enhanced responses to stress	++
+ or ++	Antidepressant response	++

[a] + = Weak association; ++ = strong association; +++ = very strong association.

6. Summary

As illustrated in Table 2, there are many similarities between FSL rats and depressed humans: reduced activity and body wt, sleep abnormalities, and increased responses to stress and to cholinergic agonists. Although there is much information indicating that the cholinergic supersensitivity in the FSL rats is associated with increases in the concentration of brain mAChRs, the data for humans are inconclusive, possibly because the appropriate brain regions have not been studied. The data for the FSL rats indicate that the elevations in mAChR occur in selected brain regions, so there is a need for more extensive receptor-binding studies in human brain.

Recent experiments suggest that changes in noncholinergic neurotransmitter systems have also occurred during the pharmacogenetic selection of the FSL rats (Table 1). However, there is insufficient information at present to determine whether these changes have occurred as a result of a primary change in the cholinergic system or because of some other factors. Although these findings do not permit a simple statement about relations between the cholinergic system and depressive disorders, they are consistent with the growing literature indicating multiple neurochemical changes in depressed humans.

To date, a limited number of antidepressants have been used in the FSL rats, unlike the case with the olfactory bulbectomy model (*see* the chapter by Richardson in this volume). The fact that the FSL rats have not responded to all the antidepressants attempted could be taken as evidence against their utility as an animal model of depression. However, given the generally accepted notion that depressive disorders are heterogeneous, it would be unusual and unexpected if the FSL rats responded to all clinically useful antidepressants; after all, depressive humans do not all respond to any single antidepressant. Instead, therefore, we view the evidence of the FSL rats responding to some antidepressants as support for their utility as an animal model of depression. Additional work with antidepressants having selective modes of action could clarify the basis for the "depressive" tendencies in the FSL rats.

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Methods of Assessing Circadian Rhythms in Animal Models of Affective Disorders

Naoto Yamada and Saburo Takahashi

1. Introduction

The existence of circadian rhythms, near 24-hour patterns of variation in biological functions, has been well established in many species. There is currently a great deal of interest in whether or not disturbed circadian rhythms are involved in the pathogenesis or etiology of some types of psychiatric disorders. Indeed, several types of sleep disorders, such as delayed sleep phase syndrome (Weitzman et al., 1981; Czeisler et al., 1981), advanced sleep phase syndrome (Moldofsky et al., 1986), non-24-hour sleep–wake syndrome (Miles et al., 1977; Kokkoris et al., 1978; Weber et al., 1980), and narcolepsy (Moskoetal., 1983), are considered to be diseases based on disturbed circadian rhythms.

Besides these sleep disorders, disturbed circadian rhythms also have been reported in affective disorders, and have been a topic of interest to researchers and clinicians in psychiatry (reviewed in Hallonquist et al., 1986; Healy, 1987). Clinical features of depression, such as early morning awakening, diurnal variations with morning worsening of mood, seasonality of illness, or the cyclic character of the phase of illness, are evidence that disturbed circadian rhythms are involved in the disease (Wehr and Goodwin, 1981). In addition, recent research in chronobiology has pointed out that several physiological functions in depressed patients, for example, body temperature, rapid eye

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movement (REM) latency, heart rate, motor activity, plasma and urinary metabolites of neurotransmitters, or cortisol and melatonin secretion, exhibit abnormalities in their circadian rhythms, (reviewed in Wehr and Goodwin, 1981). Furthermore, some chemical substances, including tricyclic antidepressants, lithium, and monoamine oxidase inhibitors, which are used as antidepressant or antimanic drugs, affect the circadian rhythm of a variety of physiological functions (Wirz-Justice, 1983), confirming the hypothesis that disturbed circadian rhythms are involved in affective disorders. More recently, a particular pattern of recurrent depression, seasonal affective disorder (SAD), has drawn the attention of scientists. SAD is characterized by recurrent cycles of fall–winter depression and spring–summer hypomania (or euthymia) (Rosenthal et al., 1984). Although the disorder is characterized by a circannual pattern of symptoms, disturbed circadian rhythms in various physiological functions have been found in the disorder (Lewy et al., 1988; Skwerer et al., 1988). Analysis of these circadian rhythm abnormalities may elucidate underlying mechanisms of this disorder.

There are many kinds of animal models of affective disorders used to investigate further the pathophysiology and etiology of these disorders (Jesberger and Richardson, 1985). Although both neurochemical and behavioral aspects of the models for affective disorders have been well investigated, little attention has been paid to the chronobiological aspect of the models. Therefore, in this chapter, we intend to review some representatives of chronobiological hypotheses for affective disorders, and to describe how to measure and assess the circadian rhythm of selected animal models of affective disorders.

2. Basic Concepts of Circadian Rhythm

First of all, some parameters of circadian rhythms and basic chronobiological concepts are explained.

2.1. Explanation of Rhythm Parameters

Various parameters characteristic of circadian rhythms are of importance, that is phase, amplitude, and mesor. In order to explain and visualize these parameters, an example of body

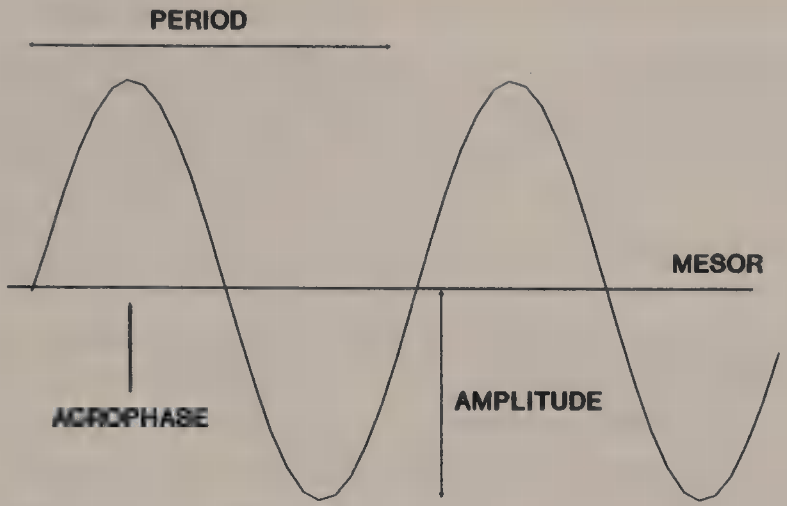

Fig. 1. Rhythm parameters of period, phase, amplitude, and mesor of body temperature rhythm obtained from Cosinor analysis.

temperature rhythm in a human is shown in Fig. 1. The period is the duration of one complete cycle. The amplitude can be defined as the range of the oscillation, and the mesor as the average value of the variable during one cycle. The phase can refer to the location of any particular point in the waveform of the rhythm, and acrophase means the timing of its peak. In order to estimate the period, "reference phase" (clear and stable phase position of circadian rhythm) is used. As the reference phase, the acrophase, onset time, or offset time are commonly used.

Because a biological rhythm is an expression of a certain physiological function, generated by oscillators within the organism, different waveforms of the rhythms observed depend on different physiological functions (Minors and Waterhouse, 1988). In other words, all biological rhythms differ in their shape, amplitude, mesor, and acrophase. For example, the waveform of body temperature looks like a sinusoidal curve, but activity rhythm resembles a square one. Furthermore, some kinds of hormones have a pulsate secretion, making the waveform more complex. Both the amplitude and the mesor are easily influenced by environmental stimuli, and moreover, are difficult to analyze from the data of some kinds of biological rhythms, such as the sleep–wake cycle, which is expressed as "on" and "off." In order to analyze the biological rhythm, therefore, it is very important to take the waveform of the biological rhythm into consideration.

2.2. Basic Concepts of Human Circadian Rhythm

"Overt rhythms," that is, observed biological rhythms, are assumed to be generated by "oscillators" within the organism that are capable of measuring time and are self-sustained, even when free of an external time cue, or zeitgeber (Moore-Ede et al., 1976). The number of oscillators differ with the species. In mammals, there is considerable evidence that the suprachiasmatic nucleus (SCN) is a primary oscillator generating circadian rhythms (Rusak and Zucker, 1979). In general, human circadian systems are thought to consist of two oscillators, namely a strong oscillator and a weak oscillator (Wever, 1979; Kronauer et al., 1982; Moore-Ede, 1983; Moore-Ede et al., 1983). The former, which controls circadian rhythms of body temperature, REM sleep propensity, and cortisol secretion, is endogenous. In contrast, the latter, which controls the sleep–wake cycle and some sleep-related hormones, is easily synchronized or entrained to environmental stimuli, such as light–dark and temperature cycle, or psychosocial factors. Ordinarily, the strong oscillator, which free-runs with a period longer than 24 h (approx 25 h), in the absence of environmental time cues, is coupled to the weak one, which is entrained to 24 h, by environmental time cues, resulting in 24-h biological rhythms. Under conditions free of the time cues, however, a few individuals eventually show separate circadian rhythms between the strong and the weak oscillators; that is called internal desynchronization, probably because the strong oscillator free-runs faster than the weak one (Wever, 1979; Czeisler et al., 1980).

3. Factors Affecting Biological Rhythms

There are several factors that affect biological rhythms, acting directly on the circadian oscillators (a zeitgeber effect) or having no effect on them, but affecting the biological rhythm (a masking effect).

3.1. Environmental Conditions

There are several environmental time cues. Some of them influence the biological rhythms by acting on the oscillators;

others directly influence the biological rhythms without acting on the oscillators. Because they make the expressed biological rhythm more complicated, it is important for investigators not only to know what kinds of factors could affect the biological rhythms, but also to take such factors (i.e., temporal isolation) into account.

3.1.1. Light

The light–dark cycle is the strongest recurring time cue, affecting the circadian rhythms in both animals and plants (Aschoff, 1960). Investigators should therefore pay special attention to it, particularly in chronobiological studies. In animal studies, even a weak dim light pulse of 5 lx for 1 s is reported to be enough to affect the phase of circadian rhythms (DeCoursey, 1972), so that either a sealed box or room, into which light is unable to penetrate, is needed to study a free-running period. In addition, under the condition of constant light, the difference in light intensity also affects the circadian rhythms. Aschoff's rule proposes a relationship between light intensity and the free-running period, and states that light intensity lengthens the period in nocturnal animals, and shortens it in diurnal animals (Aschoff, 1958,1960; Pittendrigh, 1960; Hoffmann, 1965). A light source commonly used is a white fluorescent tube or incandescent lamp. In order to take away the aftereffect of previous light exposure, two weeks are needed for acclimatization of the animal before any biological rhythm data can be collected.

3.1.2. Temperature

Temperature cycle is a secondary time cue, which is capable of affecting the biological rhythm (Underwood, 1985). However, the effect of temperature cycle on circadian rhythms is complex. Temperature cycle has been shown to synchronize the biological rhythms in some animals (Tokura and Aschoff, 1983; Aschoff and Tokura, 1986), whereas it has no effect in others (Bruce, 1960; Sulzman et al., 1977). A large amplitude of temperature cycle has been shown to synchronize the biological rhythms (Bruce, 1960), whereas a smaller one has no effect. In addition, it has been shown that the free-running period of the biological rhythms in diurnal animals shortens with increasing temperature, whereas it lengthens in nocturnal animals (Aschoff, 1979).

3.1.3. Other Environmental Factors

Both noise and humidity changes do not appear to synchronize the circadian oscillator, but rather, have masking effects on the circadian rhythm. Therefore, these experimental conditions should be kept constant in temporal isolation experiments.

3.1.4. Our Experimental Conditions

Consideration of the above synchronizing and masking environmental time cues requires specialized conditions for chronobiological studies. The photograph shows our experimental condition (Fig. 2). All of our chronobiological experiments have been performed in these sealed boxes, into which external time cues, such as light and noise, are prevented from penetrating. Air is ventilated automatically into the sealed boxes 50×/min. Other environmental conditions (i.e., temperature and humidity) are also kept constant. The lighting condition of each box is variable, in accordance with the experimental design.

3.1.5. Ocular Enucleation

In order to completely prevent the light effect on circadian rhythms, or to conduct the experiments under uncontrolled lighting conditions, the ocular enucleation procedure is commonly used. The eyelids of the animals, which are found to be closed a few days after birth, are opened at the line of adult separation with a transverse incision, and then the globe is pushed forward and elevated by application of pressure at the lateral orbital margin to allow for cutting the optic nerve at its junction with the globe (Krieger, 1973). This procedure has been shown to prevent light cues from affecting the circadian rhythms of physiological functions in animals.

3.2. Feeding Conditions

Feeding schedules also have been shown to synchronize behavioral rhythm in many mammals (Boulos and Terman, 1980). In experiments on temporal isolation, an ad libitum feed-

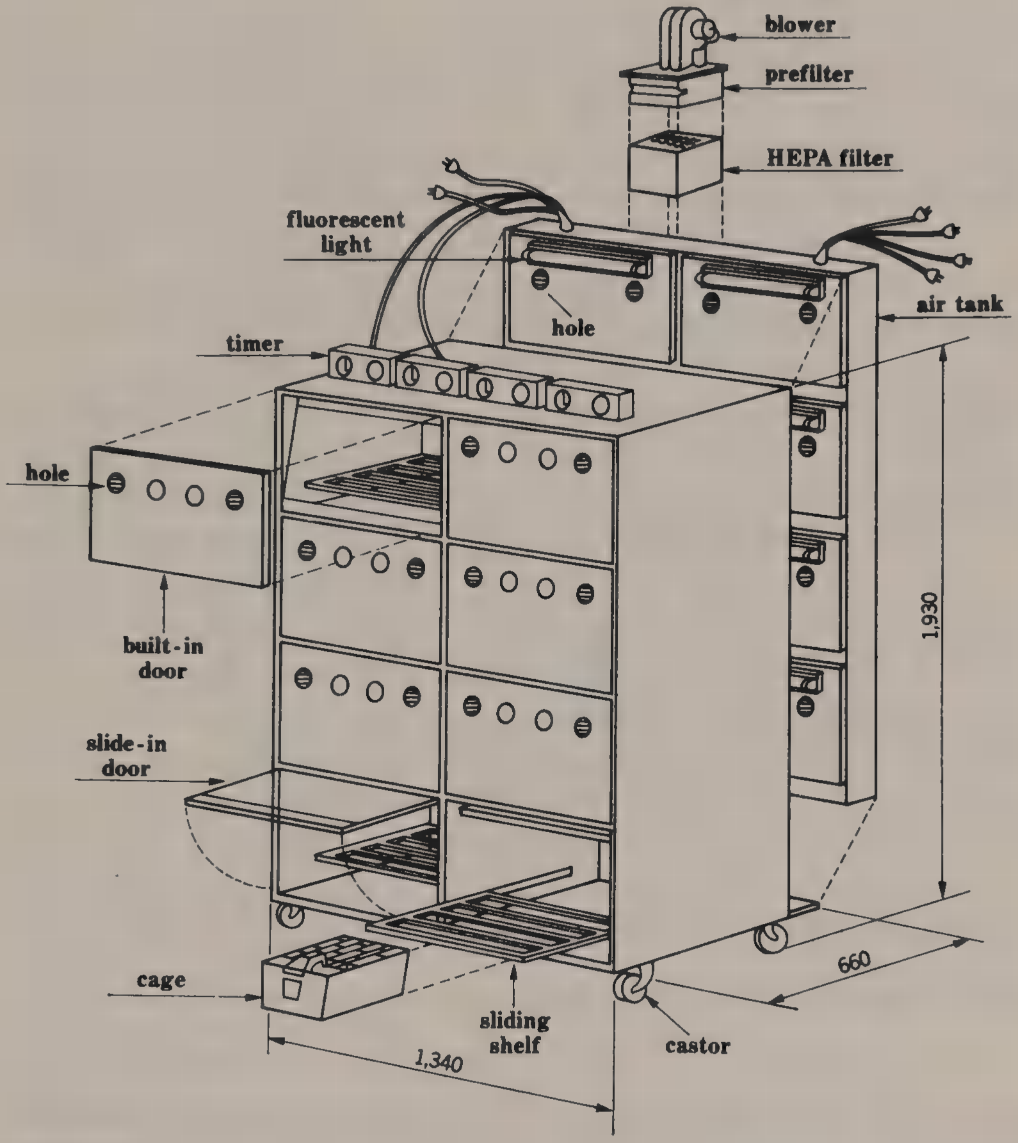

Fig. 2. Structural outline of our experimental rack. Each rack consists of eight boxes. Environmental conditions, such as temperature and humidity, are kept constant. The lighting condition of each box is variable, in accordance with the experimental design.

ing schedule is usually used. A restricted feeding schedule is one of the useful methods for researchers to investigate the entrained mechanism in chronobiology, but whether it directly affects the biological rhythms needs further investigation (Krieger et al., 1977; Stephan et al., 1979; Boulos et al., 1980). The drinking schedule has the same effect on the biological rhythms (Johnson and Levine, 1973).

3.3. Chemical Substances

Under conditions free of time cues, and under constant light, temperature, and humidity, the period of the free-running rhythm is believed to be stable. In recent years, however, there have been several reports concerning factors that change the free-running period, even under constant circumstances. Some chemical substances, including lithium (Engelmann, 1973; Maurer and Engelmann, 1974; Richter, 1977; Hofmann et al., 1978; Kripke and Wyborney, 1980; Campbell et al., 1988), tricyclic antidepressants, and monoamine oxidase inhibitors (Wirz-Justice and Campbell, 1982), have been extensively studied and shown to lengthen the free-running period. On the contrary, zotepine, which has a potent ability to decrease serotonin as well as dopamine in the brain, and is used as an antimanic drug in Japan, decreases the free-running period of activity (Minowada et al., 1988). Furthermore, benzodiazepines have been shown to phase-shift the circadian rhythm (Turek and Losee-Olson, 1986, 1987a,b). Besides these chemical substances commonly used in psychiatric treatments, other substances, including heavy water (Bruce and Pittendrigh, 1960; Daan and Pittendrigh, 1976b), and some peptides (Albers et al., 1984; Albers and Ferris, 1984) have also been reported to affect circadian rhythms (Fig. 3).

3.4. Aging, Species, and the Other Factors

The free-running period of biological rhythms differs with species (Pittendrigh and Daan, 1976a). In general, the free-running period is longer than 24 h in rats and is shorter than 24 h in hamsters.

Regarding aging, Pittendrigh and Daan (1974) reported that the period of free-running activity rhythm was shorter in old hamsters than in younger ones. Other researches have also confirmed these findings (Morin, 1988).

In humans, social factors, rather than light, are suggested to be the most important time cues (Aschoff et al., 1975). It has been reported that, compared to individually housed animals, phases of group-housed animals have a tendency to exhibit convergence in the timing of acrophase in adrenocortical activity rhythm, indicating that a social factor associated with group housing may also act as a time cue (Takahashi et al., 1978).

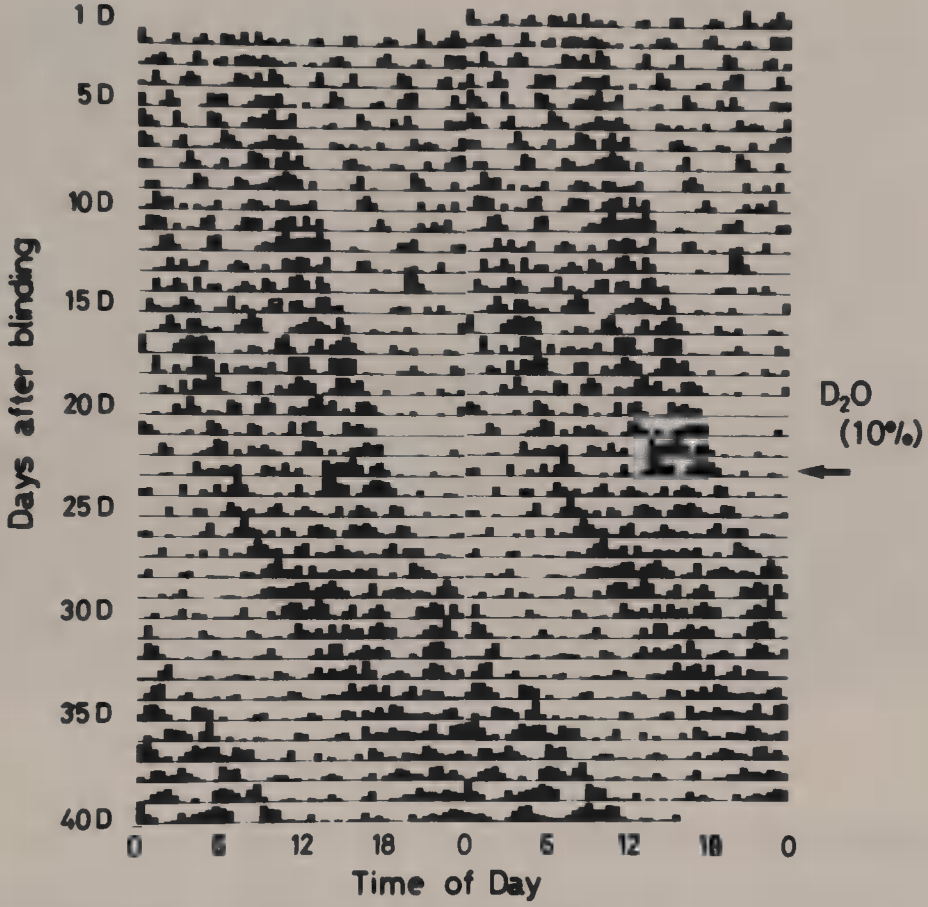

Fig. 3. The effect of D_2O on the free-running period of locomotor activity of the rat. The actogram is shown by a double-plotted method. An arrow shows the start time of administration of the drug. After administration, the free-running period of locomotor activity is elongated.

Differences in tools for determination of the activity rhythm (i.e., access to wheel or not) also have been shown to affect circadian rhythms of hamsters (Aschoff et al., 1973; Pratt and Goldman, 1986) and rats (Yamada et al., 1986,1988). This will be described more fully later.

4. What Kinds of Biological Rhythms Should Be Used for Determination of Circadian Rhythms?

Both long-term observation and frequent consecutive sampling are needed to calculate rhythm parameters. It is therefore very important to select the appropriate biological rhythms. The following biological rhythms are commonly used.

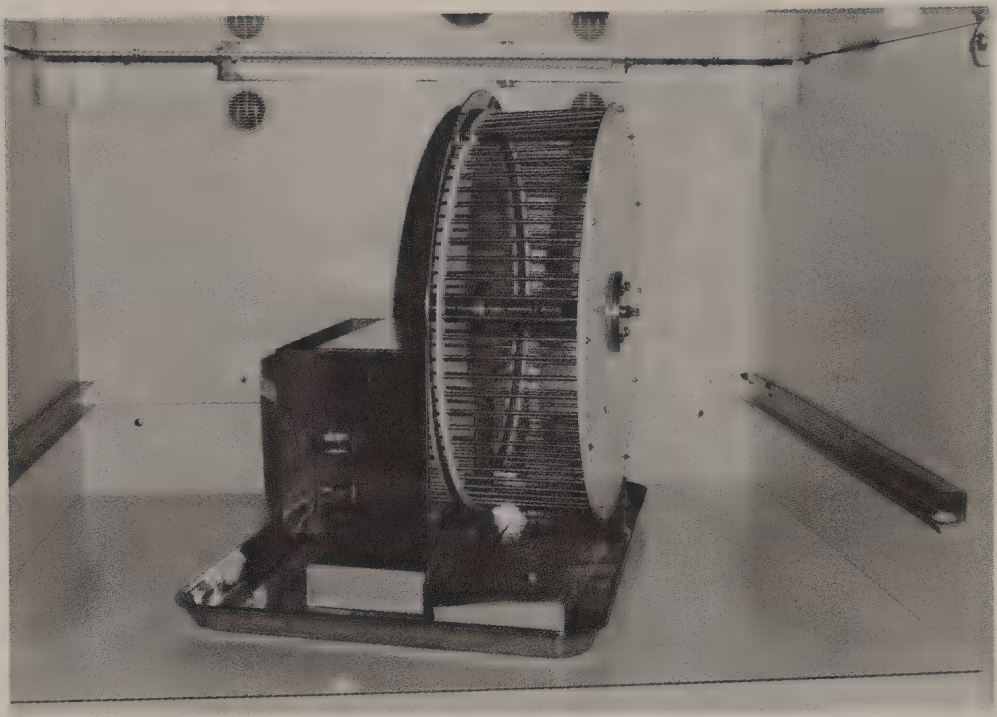

Fig. 4. Running wheel.

4.1. Behavior Rhythm

Behavior rhythms are frequently used in studies of circadian rhythms in animals, simply because they exhibit clear-cut rhythm patterns and are easily monitored under laboratory conditions. Rhythms are commonly assessed for ambulation, wheel-running, hopping, lever-pressing, feeding, micturition, drinking, and sexual behaviors. Either spontaneous or learned behaviors may be measured.

Wheel-running activity, commonly used for measurement of the behavior rhythm of rodents, shows a clear circadian pattern. Wheel-running rhythms can be obtained by recording the number of revolutions of the wheel (Fig. 4). On the other hand, spontaneous activity is measured with a variety of devices, such as an Animex activity monitor or by measuring intersection of an infrared light beam by the animal crossing the beam (Fig. 5). Figure 6 shows the actograms of running-wheel activity and spontaneous activity as measured by an Animex type of device in a blinded rat.

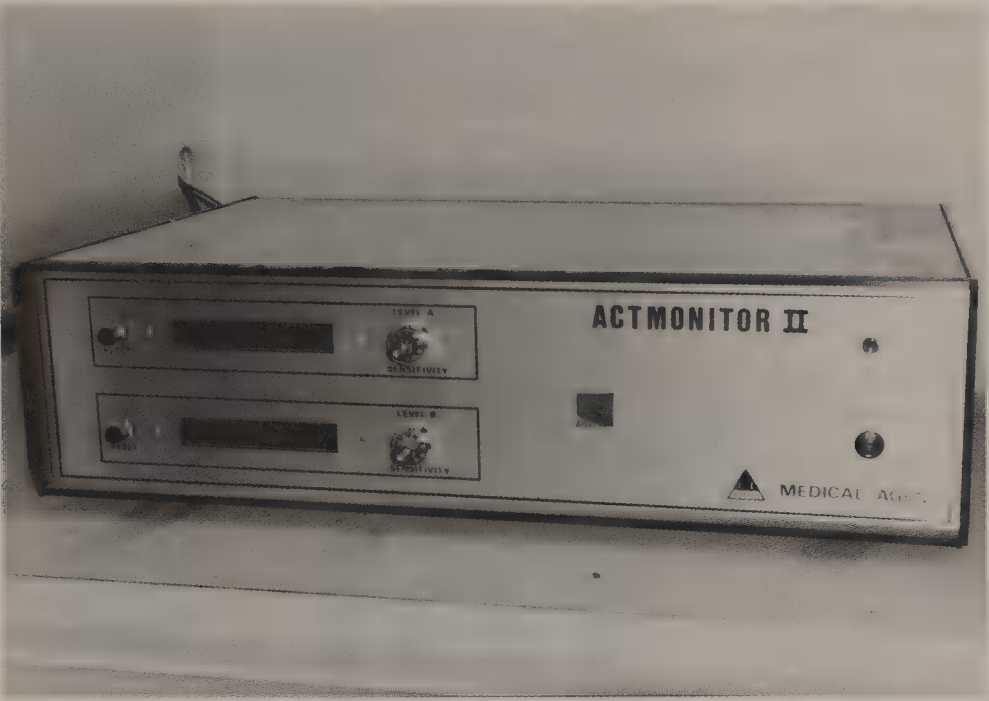

Fig. 5. Animex type of device.

Drinking also displays a clear circadian pattern. Recently, an apparatus has been developed that can measure the volume of the water intake by means of a red beam or electrical resistance (Peng and Kang, 1984).

4.1.1. Dependence of Activity Rhythms on Measurement Tools

The wheel-running activity has been misinterpreted as a measure of general activity. Recent experimental evidence suggests that this behavior has a far more specific function for the animal (Mather, 1981). Pratt and Goldman (1986) reported that the mean free-running period of hamsters housed in running wheels was less than the mean period of hamsters housed in simulated burrows, demonstrating that the activity rhythm differs with the measurement devices. Furthermore, Yamada et al. (1986, 1988) have demonstrated that the free-running period tends to be longer when it is determined with an Animex monitor than with a running wheel in blinded rats (Fig. 6). Since these two devices are commonly used to determine the behavior

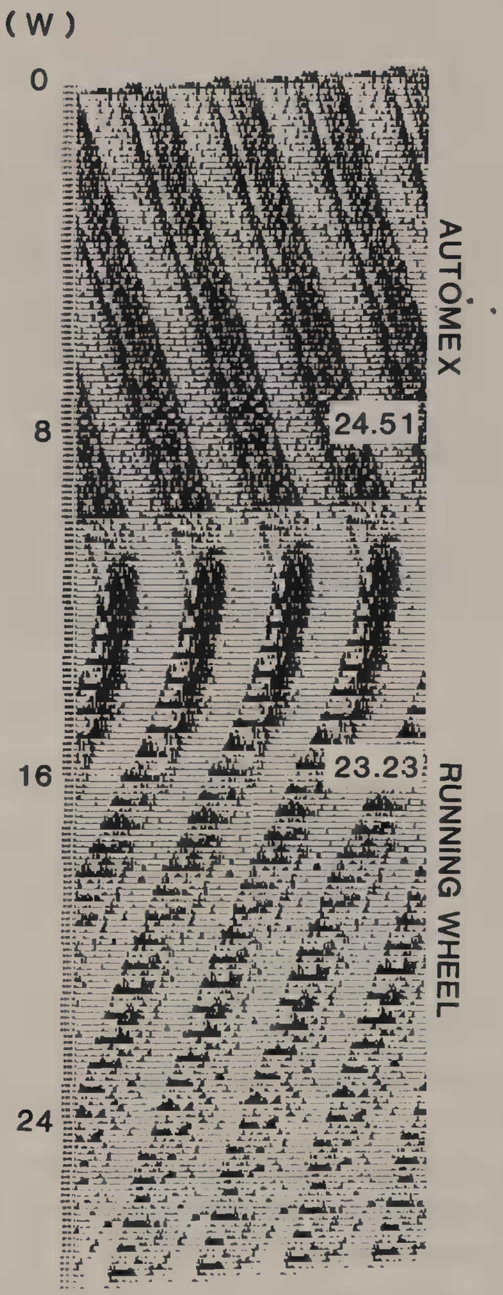

Fig. 6. Tetra-plotted actogram of activity rhythm of the rat using a running wheel and an Animex type of device. The free-running period became longer when it was determined with an Animex, whereas it became shorter when determined with a running wheel.

rhythm, these results suggest that caution is required in selecting the device with which to determine the activity rhythm.

4.2. Body Temperature

Body temperature is widely used to study circadian rhythms, simply because it not only shows clear waveforms of circadian rhythm, but is also easy to measure consecutively on a chronic basis. Furthermore, because the waveform of body temperature resembles a sinusoid curve, Cosinor analysis of such data is both convenient and appropriate. In general, body temperature is measured using a telethermometer surgically implanted in subcutaneous tissue, the peritoneal cavity, or the brain, or alternatively, using a rectal probe (Reite and Short, 1983).

4.3. Neuronal Activity

Both electroencephalograph recordings commonly used for determining the sleep–wake cycle of mammals and neuronal activity used for the purpose of analyzing circadian mechanisms in the central nervous system display circadian rhythms. Because of the difficulty of long-term observation, as well as the restriction of behavior with these procedures, their utility is limited.

4.4. Hormone Activity

A variety of hormones show circadian rhythms with pulsative secretion. Some of them, including cortisol and melatonin, are assumed to be generated by the strong oscillator, and others by the weak one. In order to analyze the parameters of the circadian rhythms, successive samples, called serially dependent samples (serial sampling of individual animals), are essential, because serially independent samples (group data) could mask a cyclic function by algebraic summation and averaging of sampling data. In humans or large animals, numerous successive blood samples for assay are easily obtained through a catheter inserted in a large vein. On the other hand, because of the difficulty of successive sampling from freely moving small animals, such as rats and hamsters, a special method, the tail-tip incision method, is used; the tail end is cut by razor, and a few drops of blood are collected into a heparinized capillary tube (Krieger, 1973; Takahashi et al., 1979).

4.5. Biochemical Substances

Urinary excretion of electrolytes, 5-hydroxyindole-3-acetic acid, homovanillic acid, free tryptophan, tyrosine, and 3-methoxy-4-hydroxyphenylglycol, as well as serum serotonin, tyrosine, and tryptophan, has been reported to show circadian rhythms (Wehr and Goodwin, 1983). In these cases, it is essential to take serially dependent samples.

5. Techniques for Analysis

The choice of which type of techniques should be used for the analysis of the rhythm parameters depends on the waveform of the biological rhythms, because the results can be misinterpreted unless the analytical method fits the form of biological rhythm. For example, Cosinor analysis is usually used to analyze body temperature rhythm because of its similarity to a sinusoidal curve, whereas this method of analysis should not be adopted to estimate other rhythms which waveforms are different from a sinusoidal curve.

5.1. Visual Inspection

A biological rhythm that displays a clear-cut reference phase is suitable for visual inspection. In this case, an actogram should be drawn to estimate the period of biological rhythms. Then, an eye-fitted line is drawn on the actogram, with respect to the reference phase. Either onset time or offset time is commonly used as the reference phase in the case of motor activity and drinking rhythms. In the case of the Animex record, the free-running period should be determined from the offset time, because the offset time of the activity phase is more stable and clear-cut than the onset time. On the other hand, in the case of running-wheel records, it should be determined from the onset time, because the onset time of the activity phase is more abrupt (Takahashi et al., 1984).

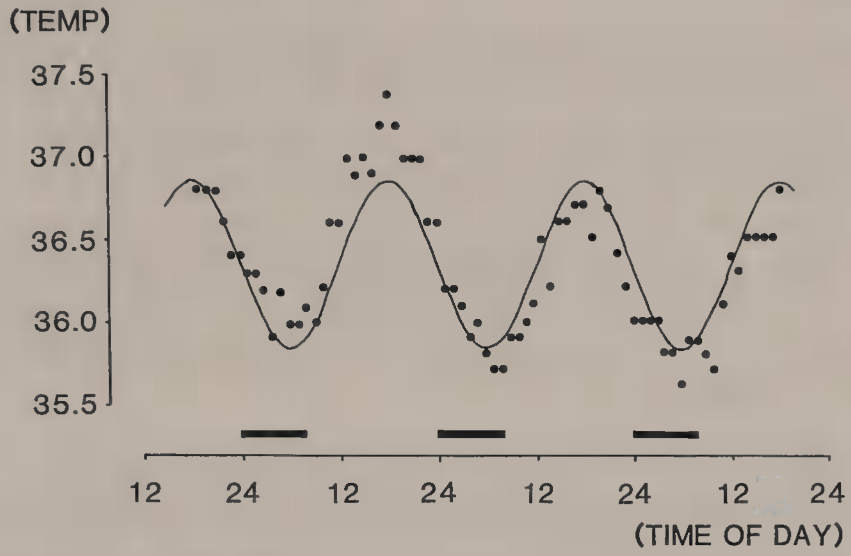

Fig. 7. Cosinor analysis of human body temperature rhythm.

5.2. Mathematical and Statistical Analysis

Mathematical and statistical analysis should be applied after inspecting the data visually. As previously mentioned, the choice of the correct method for analysis is important. For example, analysis using power spectrum or maximum entropy methods does not contain information about the phase. In addition, cosinor analysis should not be used to estimate biological rhythms with a waveform different from a sinusoidal curve. Furthermore, very short observation periods cannot give proper estimations for circadian parameters in any analytical methods. Programs for personal computers are commercially available.

5.2.1. Cosinor Analysis (Least Square Method)

This analytical technique proposed by Halberg is based on a best-fitting cosine function. Namely, it fits the data to a theoretical curve as

$$y = M + A \cos (wt + \phi)$$

where M = mesor, A = amplitude, w = radical frequency, and ϕ= acrophase of the circadian rhythm. For example, Fig. 7 shows the cosine curve fitting the data of human body temperature rhythm. This technique is probably the most frequently used mathematical analysis of all because of the advantage of its potential to calculate parameters, such as acrophase, amplitude, and period, from a sparse and irregular sampling (Monk, 1987). The results calculated can, however, be misleading if the biological rhythm has a waveform that is quite different from a sinusoidal curve or if the sampling times are too irregular (e.g., when only a night or day period is used for calculation). The sinusoid fitting procedure is described in detail elsewhere (Halberg et al., 1972; Nelson et al., 1979; Monk and Fort, 1983; Spencer, 1989).

5.2.2. Power Spectrum

This method is used for determining the period of various biological rhythms. It relies on the Fourier series. By means of this, a given time series from any complex waves may be represented as

$$\begin{aligned} f(t) &= a_0/2 + (a_1\cos wt + b_1\sin wt) \\ &\quad + (a_2\cos 2wt + b_2 \sin 2wt) \\ &\quad + (a_3\cos 3wt + b_3\sin 3wt) + \ldots \end{aligned}$$

and the amplitude as $A = (a^2 + b^2)^{1/2}$. The power spectrum is obtained by plotting frequency (horizontal axis) vs square of the amplitude (vertical axis). Recent advances in personal computers make it possible to calculate a large volume of data rapidly. In this case, the Fast Fourier algorithm is available. The procedure is described in detail elsewhere (Brigham, 1974; Dirlich et al., 1981; Spencer, 1989).

Although this method is excellent, the results are misleading when the observation period is too short. For example, the frequency resolution depends on the length of the observation interval, and the reliability of the spectral representation is limited by sampling interval: components with periods of not less than 6 h are reliably measured using a sampling interval of 3 h. Furthermore, rhythm parameters other than the period cannot be obtained with this method.

5.2.3. Maximum Entropy Method

The maximum entropy method advocated by Burg was developed from the concept of maximizing the expected entropy

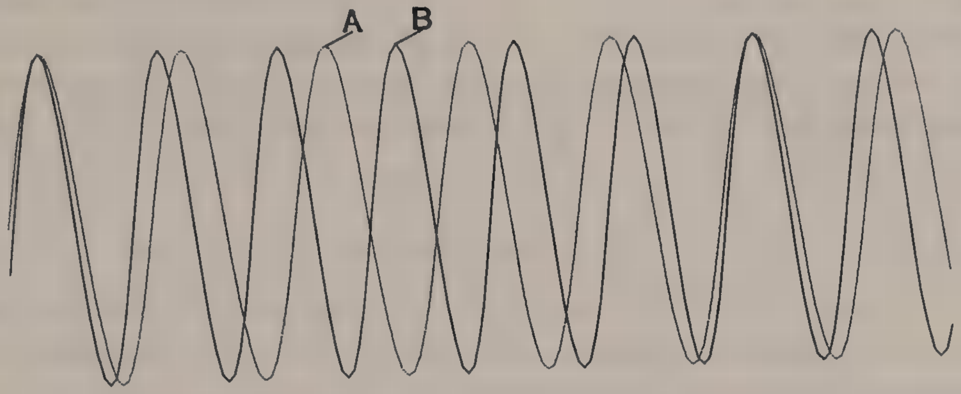

Fig. 8. Hypothetical model of the free-running hypothesis of cyclic mood disorder. A and B show a weak oscillator and a strong one, respectively.

(Hino, 1986). The advantages of this method are the obtaining of reliable results from short observation periods and the high resolution of the frequency domain, in comparison with the other methods, such as power spectrum (Radoski et al., 1975; Malbecq and De Prins, 1981).

6. Chronobiological Hypothesis for Affective Disorders

As mentioned above, recent findings suggest the possibility that disturbed circadian rhythms are involved in affective disorders. The following are representatives of hypotheses proposed, demonstrating that disturbed circadian rhythms may play a causal role in some affective disorders.

6.1. The Free-Running Hypothesis of Cyclic Mood Disorders

Halberg has assumed that bipolar circular manic-depressive disorder could result from internal desynchronization resulting from the uncoupling of one oscillator from another, even under the influence of the strong time cue of the 24-h light–dark cycle (Kripke et al., 1978). Figure 8 shows the hypothetical model of the circular manic-depressive. According to the hypothesis, the weak oscillator A is synchronized to the 24-h day–night cycle. On the other hand, the strong oscillator B free-runs with a period shorter than 24 h, and therefore, B periodically goes in and out of phase with A, generating the cyclic manic-depressive phenomenon. In accordance with this hypothesis, there have been several reports of disturbed circadian rhythms in patients with this disorder (Kripke et al., 1978).

6.2. The Phase-Advance Hypothesis of Depression

Wehr reanalyzed the data in the reported literature, and suggested that temperature, cortisol, and REM sleep of depressed patients, thought to be controlled by the strong oscillator, were significantly phase advanced as compared to healthy controls (Wehr and Goodwin, 1981). Figure 9 shows the theoretical schema of the phase-advance hypothesis. In contrast to the free-running hypothesis, which supposes a different free-running period between the two oscillators, this hypothesis assumes an abnormal phase position of the rhythm produced by the strong oscillator.

In support of this hypothesis, there have been many reports concerning the abnormal phase position of physiological rhythms of depressed patients (e.g., Wehr and Goodwin, 1981). Evidence contrary to the phase-advance hypothesis has also been reported (Faravelli et al., 1985; Von Zerssen et al., 1985). The pathogenetic mechanisms that could cause the advanced phase are unknown.

6.3. The Phase-Delay Hypothesis of SAD

It has been well established in animal studies that, in the constant dark condition, a single light pulse is capable of shifting the phase of circadian rhythm, depending on the timing of light exposure during the subjective day (Daan and Pittendrigh, 1976a). Exposure to light during the latter part of subjective night causes a phase advance, whereas exposure to light during the first part of subjective night causes a phase delay. The relationship between the timing of light exposure and the resulting phase shift of circadian rhythms is expressed in terms of a phase–response curve (PRC) (Daan and Pittendrigh, 1976a,b; Pittendrigh and Daan, 1976b). In psychiatric studies, several researchers have recently reported that exposure to high-intensity artificial light has antidepressant effects for some symptoms of SAD. Further-

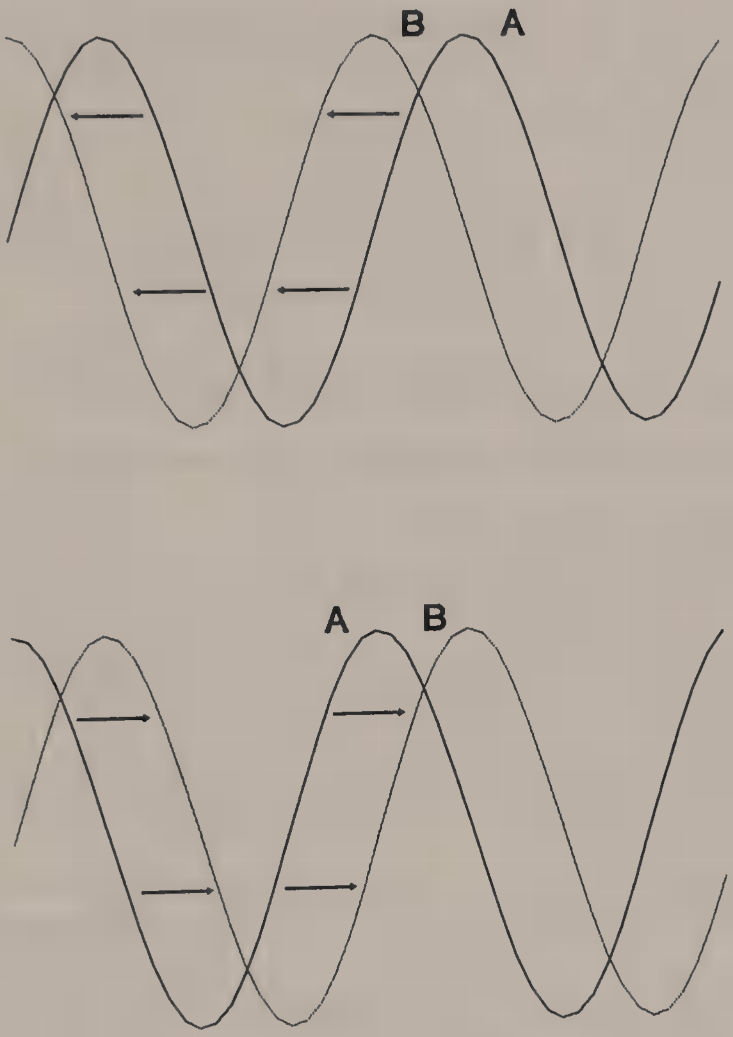

Fig. 9 A. Schematic representation of the phase-advance hypothesis for depression. According to the hypothesis, the phase of a strong oscillator is advanced against a weak oscillator, resulting in depression. B. Schematic representation of the phase-delayed hypothesis for SAD. According to the hypothesis, the phase of a strong oscillator is delayed against a weak oscillator, resulting in SAD.

more, some of these have pointed out the difference in therapeutic efficacy in terms of the different timings of light exposure; morning bright light is more effective than evening bright light.

Based on both animal studies and results obtained from phototherapy, Lewy et al. have hypothesized that most patients with SAD have an abnormally delayed phase of circadian rhythms (Lewy et al., 1988), and that therapeutic efficacy may be brought on by phase advance of the circadian rhythms by means of morning bright light (Fig. 9b). Recently, Honma and Honma have demonstrated that humans also had similarly shaped PRCs (Honma and Honma, 1988). From this point of view, SAD is assumed to be a disorder that relates to seasonal changes in circadian rhythm (Lewy et al., 1987). This is controversial. Some report timing does not matter (Hellekson et al., 1986; Jacobsen et al., 1987; James et al., 1985).

7. Chronobiological Features of Affective Disorders

7.1. Chronobiological Features of Depression

Body temperature is noninvasive and easy to measure, and many researchers have reported abnormalities in this physiological variable in depression. Regrading the period of the temperature rhythm, there is evidence for the desynchronization hypothesis, that is, the period is shorter than 24 h (Kripke et al., 1978). Others have not found evidence for the hypothesis (Pflug et al., 1976; Kripke et al., 1979; Von Zerssen et al., 1985). Concerning the phase of the temperature rhythm, the results have been controversial, because of methodological differences in each experiment. Again, there has been some evidence for the phase-advance hypothesis (Pflug et al., 1976; Kripke et al., 1978; Wehr et al., 1980) and also evidence against the hypothesis (Avery et al., 1982; Kripke et al., 1979; Faravelli et al., 1985; Von Zerssen et al., 1985). With respect to amplitude and mesor (mean temperature), somewhat more consistent results have been obtained. The amplitude in depressed patients is significantly less than in controls and tends to increase with recovery (Nikitpoulou and Crammer, 1976; Avery et al., 1982; Von Zerssen et al., 1985; Yamada et al., 1989), although there are a couple of negative reports (Kripke et al., 1979; Mellerup et al., 1978). The reduced amplitude in depressed patients is related to the higher temperature minima, not to the lower maximum levels (Avery et al., 1982). The mean temperature of depressed patients is higher than that of controls (Pflug et al., 1976; Avery et al., 1982; Von Zerssen et al., 1985).

Second, with respect to sleep–wake rhythms, Kupfer (1976) found a short REM latency in depressed patients, which is interpreted as evidence of the phase-advance hypothesis. This result has been confirmed by many authors (Gillin et al., 1979; Wehr and Goodwin, 1981; Avery et al., 1986; Schulz et al., 1978). In addition to this finding, both a long first REM period and frequent occurrence of wakefulness during the last part of sleep support the hypothesis, because these phenomena are found in normal people whose sleep period is advanced by several hours (Kupfer, 1976; Wehr and Goodwin, 1983; Schulz et al., 1978).

Third, there is relevant data from measurements of cortisol, the most frequently studied hormone in chronobiology. Since Doig et al. (1966) first reported that the circadian rhythm pattern of secretion of cortisol in depressed patients was shifted to the left (that is, phase advance), a large number of studies have confirmed this finding (Yamaguchi et al., 1978; Pfohl et al., 1985). Regarding other parameters of the circadian rhythm of cortisol secretion, consistent findings of both high amplitude and mesor in cortisol rhythm have been obtained, as demonstrated in a large number of studies in relation to the dexamethasone suppression test for the affective disorders (Carroll et al., 1976a,b, 1981).

Circadian rhythms of neurotransmitters also exhibited advances of their phases. Urinary levels of serotonin, dopamine, noradrenaline, and their metabolites were reported to phase advance (Wehr and Goodwin, 1981). However, there were insufficient data points collected in this study to determine the pattern of the circadian rhythms reliably. With respect to motor activity, decreased amplitude in depressed patients compared with normal control subjects has been reported (Wolff et al., 1985).

7.2. Chronobiological Features of SAD

SAD is characterized by annually occurring fall–winter depression and spring–summer hypomania (or euthymia). Basically, depressive symptoms may be associated with increased somnolence, fatigue, weight gain and carbohydrate craving (Rosenthal et al., 1984; Garvey et al., 1988; Blehar and Rosenthal, 1989). These symptoms have been successfully treated by artificial bright light (Lewy et al., 1982, 1987; Rosenthal et al., 1985; James et al., 1985; Yerevanian et al., 1986; Wirz-Justice et al., 1986; Wehr et al., 1986, 1987; Jacobsen et al., 1987; Isaacs et al., 1988; Rosenthal et al., 1988; Grota et al., 1989). From the studies demonstrating that melatonin is related to the exhibition of circannual changes in animals, this hormone has been well investigated in patients with SAD. Lewy et al. (1988) reported that there was a phase delay in dim light melatonin onset, and proposed the phase-delay hypothesis for SAD. Terman et al. (1987) also reported a 2-h phase advance of the melatonin circadian rhythm in patients with SAD after their recovery. On the contrary, some authors did not find any phase advance or delay of the melatonin circadian rhythm in SAD (Rosenthal et al., 1987; Skwerer et al., 1988).

8. Animal Models of Affective Disorders

The validity of animal models in relation to affective disorders has been traditionally assessed using a set of criteria proposed by Mckinney and Bunney (1969):

1. The behavioral manifestations of the syndrome should be similar to those seen in the human condition;
2. The behavioral changes should be able to be objectively detected by independent observers in different laboratories;
3. The behavioral state induced should be persistent and generalizable;
4. Including conditions used in animals should be similar to those present in human psychopathology;
5. Treatment modalities effective in reversing human disorders should be effective in animals; and
6. There must be sufficient reference control data available on the species under study.

Of a set of criteria, of course, the obvious similarity between the model and disorder is considered to be the most necessary criterion. In this connection, it is important to take the disturbed circadian rhythm into consideration as well as neurochemical and behavioral similarities.

8.1. Animal Model of Depression

Various types of animal models of affective disorders have been proposed. In general, animal models of depression could be classified into two major groups; one is produced by pharmacological interventions and the other is a stress model (Katz, 1981). The former is induced by chronic administration of chemical substances, such as reserpine, α-methyl-DOPA, 5-hydroxytryptophan, and tetrabenazine. The latter is induced by application of various stressors. Although the effects of inescapable or uncontrollable stress and of early separation have been widely investigated in terms of neurochemistry and behavior, only a few studies have investigated chronobiological characteristics of these animal models.

A number of abnormal rhythms of body temperature, heart rate, and REM latency were also reported in young monkeys in the behavioral dispair phase after separation from their mothers (Reite et al., 1982; Reite and Short, 1983). Changes in circadian rhythms of physiological functions, however, were not completely in accord with the changes found in depression; the amplitude of body temperature increased and the mesor decreased in the animal model, whereas the amplitude decreased and the mesor increased in depressed patients. REM latency increased in the animal, whereas it decreased in depressed patients. Takahashi et al. (1988) also demonstrated that stress induced by exposure to cold water lengthened the free-running period of activity rhythm in rats.

From a chronobiological point of view, on the other hand, the SCN-lesioned animal may be postulated as the most specific behavior model of depression. The SCN-lesioned rats show a similar tendency in biological rhythms to that shown by depressed patients. For example, with respect to the temperature rhythm in SCN-lesioned rats, advanced phase, decreased amplitude, and shortened free-running rhythm have been demonstrated (Powell et al., 1980; Ruis et al., 1987).

8.2. Animal Model of SAD

To date, many investigators have advocated hibernation as a model of SAD, because of similarities between hibernation and

SAD, i.e., seasonal changes in increased eating, weight gain, sleep, and decreased libido (reviewed in Mrosovsky, 1988). As Mrosovsky has pointed out, however, there are numerous dissimilarities in each seasonal change between SAD and hibernation. To date, there are no animal seasonal rhythms that can be adopted as suitable models for SAD.

9. Summary and Conclusions

To date, a variety of animal models of depression have been proposed not only on the basis of similarities between stress-induced behaviors in animals and certain symptoms of depression in humans, but also on the basis of the biogenic amine hypothesis of depression. In spite of the accumulation of the data concerning the disturbed circadian rhythms of depression, however, little attention has been paid to the chronobiological aspects of these models, as opposed to both neurochemical and behavioral characteristics. Insofar as disturbances of circadian rhythms are characteristic of affective disorders, this is a serious shortcoming. It is important to investigate the circadian rhythms of animal models of affective disorders, especially since the phenomenon of SAD suggests a causal relationship between affective disorders and disturbed circadian rhythms.

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Animal Models of Anxiety and the Screening and Development of Novel Anxiolytic Drugs

D. J. Sanger

1. Introduction

Anxiety is a term used to describe both a normal emotional state associated with stressful or psychologically difficult events and a pathological condition. When anxiety is chronic and is not clearly linked to well-defined events, it is generally considered abnormal and appropriate for psychological or psychiatric intervention. Although many forms of treatment have been applied, anxiety is currently most frequently treated with anxiolytic drugs.

As a clinical condition, anxiety is susceptible to the same biological analysis of its psychological and biochemical bases as are other abnormal psychological conditions. Much of the recent research related to anxiety has been concerned with the analysis of the actions of anxiolytic drugs and the search for novel, more effective agents. The techniques used, therefore, have largely been those of psychopharmacology, including attempts to develop animal models of anxiety. The purpose of the present chapter is to describe some of the behavioral tests that have been used in this research and to make some attempt to evaluate their success. The criteria that can be applied to evaluate the validity of such models are the same as those used in other branches of behavioral neuroscience. However, since the emphasis has been

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on antianxiety drugs, the major criterion has been the sensitivity of laboratory procedures to drug action (Treit, 1985). Indeed, relatively little attention has been paid to the extent to which animal tests mimic either the behavioral or neurochemical aspects of the clinical condition. The emphasis of the present discussion therefore will also be on the extent to which the behavioral procedures described are useful in analyzing the behavioral and physiological mechanisms of action of anxiolytic agents and in aiding the search for novel drugs.

2. Anxiolytic Drugs

The reason for the predominance of psychopharmacological studies in biological research on anxiety is probably the enormous success of benzodiazepines as anxiolytic drugs. Many drugs have been used for treating anxiety, but the prescription of once popular agents, such as barbiturates and meprobamate, has been almost completely superceded by benzodiazepines. A major reason for this is that benzodiazepines are not only effective, but they are very safe, although, like all drugs, they have undesirable side effects. Recent psychopharmacological research was also given further emphasis by the discovery that benzodiazepines bind to specific, high-affinity sites in the central nervous system (Squires and Braestrup, 1977; Mohler and Okada, 1977). These binding sites have recently been named ω receptors (Langer and Arbilla, 1988). Such findings led to the view that studies of the mechanisms of action of benzodiazepines might provide information about the physical basis of anxiety disorders, a belief described by Lader (1989) as involving misplaced logic. More reasonably, it was thought that knowledge concerning the mechanisms of action of benzodiazepines might allow researchers in pharmaceutical companies to design molecules that, although structurally different, might act at similar sites, but offer advantages over the benzodiazepines (Williams, 1984). In particular, it was hoped that compounds might be found that, although showing anxiolytic activity, would be devoid of other activities considered undesirable, such as sedative or muscle-relaxing effects. This research has been quite successful, since many compounds have now been described that, although acting as ligands for ω receptors, show pharmacological properties different from those classically displayed by benzodiazepines (Gardner, 1988). As yet, an anxioselective drug developed on the basis of such research has not entered into widespread clinical use, although several may do so within the next few years.

Most of the behavioral procedures to be described in this chapter were developed after barbiturates, meprobamate, and benzodiazepines had already been found to have clinical anxiolytic activity. Thus, they have, at least to some degree, been validated with these drugs. This led to the development of a range of sensitive procedures, and the behavioral pharmacology of anxiolytic drugs established a large body of relatively consistent data. This situation, however, has changed dramatically in recent years with the discovery that buspirone, a drug chemically and pharmacologically distinct from previously used anxiolytics, has clinical anxiolytic activity equivalent to that of benzodiazepines (Goa and Ward, 1986). It is important, therefore, when assessing the pharmacological validity of animal models, to evaluate their sensitivity both to classical anxiolytics and to buspirone and related agents, such as ipsapirone and gepirone, which also seem to exert clinical antianxiety effects (Traber and Glaser, 1987; Harto et al., 1988).

3. Punishment Procedures

Punishment procedures, or conflict tests as they are often called, have been very widely used with great success for assessing the effects of anxiolytic drugs. Punishment refers to the presentation of an aversive stimulus, generally a brief, mild electric shock, to an animal, contingent upon the emission of a particular behavior. It is this contingent relationship between a response, such as pressing a lever or licking a tube, and an aversive event that has apparently been of great importance in establishing the sensitivity of these methods to the effects of antianxiety drugs (Gray, 1977). The parameters of the aversive stimulus and of the contingency are manipulated by the experimenter, so that behavior is suppressed or at least greatly decreased in rate or intensity by the punishment. Anxiolyic drugs have been found on many occasions to increase substantially rates of responding suppressed by punishment.

It is often assumed that the behavior of animals tested in punishment procedures is under the control of two motivations, a positive motivation, such as hunger, thirst, or the need to explore, which tends to induce the animal to emit a response, and a negative motivation, presumably fear of the impending punishment, which decreases response output. The term conflict refers to the opposing nature of these two motivations. Under control conditions, the fear may be the stronger drive causing behavior to be suppressed, whereas, after administration of a fear-reducing drug, behavioral output will increase despite the fact that the animal will receive many more presentations of the aversive stimulus. A number of reviews have described the use of different punishment procedures for assessing drug action in some detail (Houser, 1978; McMillan, 1975). The present section, therefore, will deal with only a small number of the many tests that have been applied to the analysis of anxiolytic drug effects and will also discuss some recent work carried out with these methods.

3.1. The Four-Plate Test

This is probably one of the quickest and easiest punishment methods, and was first introduced over 20 years ago by Boissier and his colleagues (1968) following work by Slotnik and Jarvik (1966). There have been relatively few recent publications involving this test, but it is in routine use as a screening tool in some laboratories and was submitted to extensive pharmacological validation (Aron et al., 1971).

The basis of the test is that a mouse is placed in a small box, the floor of which is made up of four metal plates. The mouse naturally moves around the box, but each time it crosses from one plate to another, it is given a brief electric footshock. This punishment has the effect of greatly reducing the amount of exploration shown by the animal. If, however, a mouse is given an injection of an anxiolytic drug, the amount of exploration is greatly increased, presumably because the drug makes the punishment less aversive and thus less effective at suppressing exploration.

In the original technique, mice were tested for a very short period (1 min following 15 s of exploration without shock), which makes the four-plate test very rapid and thus suitable for screening large numbers of compounds (Stephens and Andrews, 1991). Aron et al. (1971) in fact proposed this test as a rapid screening method and were careful not to suggest that it provided a model of clinical anxiety. They pointed out that the test could effectively be used for selecting potential anxiolytics if validated pharmacologically and, therefore, presented the results of their studies with an impressively long list of anxiolytic and other drugs.

In general, drugs known to have clinical antianxiety activity (benzodiazepines, barbiturates, and carbamates) increased the number of punished crossings. However, several other anxiolytics, including two benzodiazepines, clorazepate and tetrazepam, did not show activity. In addition, although many drugs from other classes were inactive, there were a few nonanxiolytics that increased punished crossing. Several psychomotor stimulants, including amphetamine, caffeine, and cocaine, also increased box crossings, and the authors (Aron et al., 1971) suggested that separate tests of unpunished locomotion were necessary to distinguish psychomotor stimulants from anxiolytics. Several other compounds, not known to have clinical anxiolytic activity, including some anticonvulsants and antihistamines, were also active showing that, despite the advantages of speed, the four-plate test gave rise to both false positives and false negatives.

Aron et al. (1971) compared the effects of drugs on punished exploration in the test box with the actions of the same compounds on unpunished locomotion in different chambers. A better and simpler comparison would seem to be to test drugs in animals placed in the same chambers with or without shock (i.e., punished or unpunished exploration). The problem with this method is that benzodiazepines frequently increase both punished and unpunished locomotion (cf. Stephens et al., 1985 for such an effect with diazepam), indicating that, although this

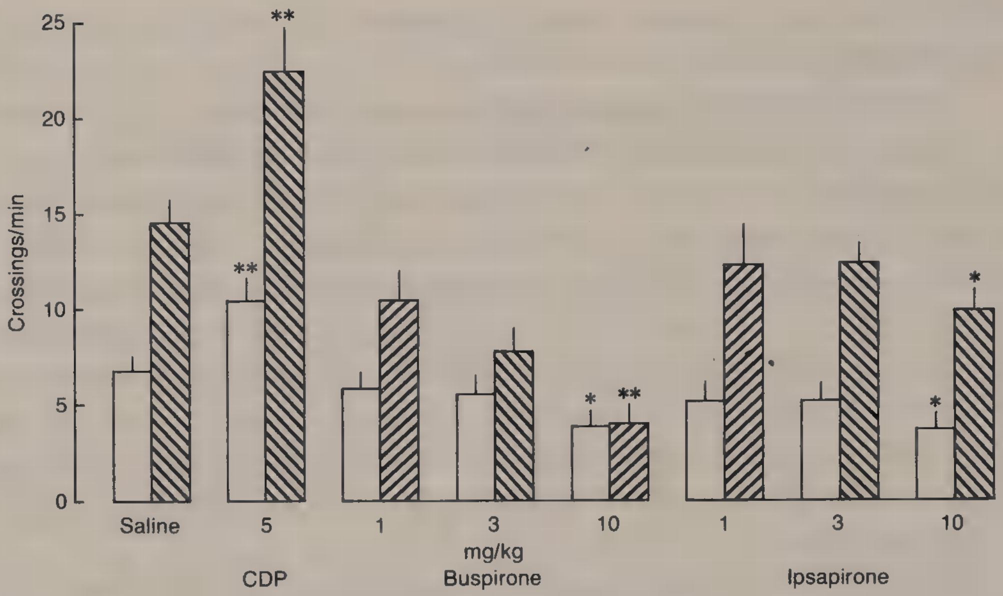

Fig. 1. The effects of a dose of chlordiazepoxide (5 mg/kg, ip) and of three doses of buspirone and ipsapirone (1, 3, 10 mg/kg, ip) on the exploratory behavior of mice measured in the four-plate test. Different animals were tested with (□) or without (▤) electric shock punishment for crossing between the four plates of the test-box floor. Chlordiazepoxide increased activity in both the punished and unpunished conditions. Buspirone and ipsapirone did not increase activity, and decreased both punished and unpunished activity at the high dose; ${}^{*}p < 0.05$; ${}^{**}p < 0.01$ difference from control animals.

procedure may be useful for screening, it does not necessarily provide a clear distinction between anxiolytics and psychomotor stimulants.

Figure 1 presents an example of such an effect where a dose of chlordiazepoxide produced a statistically significant increase in both punished and unpunished exploration. The figure also illustrates another shortcoming of this method, which is that it is not sensitive to buspirone and related drugs. Neither buspirone nor ipsapirone produced any increases in punished exploration, and both compounds decreased exploration at the highest doses.

3.2. Punished Drinking

The punishment procedure most frequently used for assessing the effects of anxiolytic drugs and, indeed, for screening for anxiolytic activity involves the suppression of drinking in thirsty rats by the presentation of mild electric shocks contingent upon contact with a drinking tube. A simple and rapid version of such a punished drinking method is often referred to as the Vogel test, after the first author of one of the early publications describing the effects of anxiolytic drugs. Vogel and coworkers (Vogel et al., 1971), following previous work (Leaf and Muller, 1965; Naess and Rasmussen, 1958), reported that chlordiazepoxide, diazepam, meprobamate, and pentobarbital increased drinking suppressed by punishment, whereas *d*-amphetamine, magnesium pemoline, and scopolamine were inactive.

The details of the method used by Vogel et al. are relatively simple and have been used successfully, with little alteration, in many other laboratories. Rats were deprived of water for 48 h and were placed in a test chamber containing a drinking spout. Whenever an animal made contact with the spout, a pulse generator sent out pulses at a rate of 7/s (to imitate the natural rate of licking of rats), and with every 20 pulses (i.e., just under 3 s of contact with the tube or 20 notional licks), the animal received a shock through the tube. In control animals, this procedure resulted in very low rates of drinking, which were substantially increased by the anxiolytic drugs. Many laboratories have replicated the findings that benzodiazepines, other ligands for the same recognition sites, barbiturates, and carbamates increased drinking (e.g., Sanger et al., 1985). Although there have been reports that analgesic drugs can increase punished drinking, such compounds are generally inactive, suggesting that the release of punished drinking produced by anxiolytics is not the result of analgesia. It has also been reported that the relative potency of different benzodiazepines in increasing punished drinking is correlated with their affinity for benzodiazepine binding sites (Malick and Enna, 1979; Nakasuka et al., 1985). This observation, together with the finding that the effects of such drugs are antagonized by benzodiazepine antagonists, such as flumazenil and CGS 8216 (Patel et al., 1983), provides strong evidence that this behavioral effect of benzodiazepines is mediated by activity at these sites.

An objection sometimes raised to the use of punished drinking as a measure of anxiolytic activity is that the drugs known to increase drinking under these conditions also increase the intake of water and other fluids induced by fluid deprivation or other manipulations (Cooper and Estall, 1985). Thus, it may be argued that the increase in punished drinking produced by benzodiazepines and barbiturates may be the result of increased thirst rather than decreased fear or anxiety. Conversely, drugs without anxiolytic activity that increase thirst may be detected as false positives in such procedures (Carli and Samanin, 1982; Leander, 1983; Patel and Malick, 1980). Although this problem has never been completely resolved, it has not prevented the widespread use of punished drinking for studying anxiolytic activity. Some researchers have developed procedures in which drug action on both punished and unpunished drinking has been assessed and, in general, anxiolytics produced greater increases in the latter (e.g., Petersen and Buus Lassen, 1981; McCown et al., 1983). It may be objected, however, that with such methods it is seldom possible to equate baselines of punished and unpunished drinking, so that differential effects might conceivably be baseline-dependent.

The effects of buspirone on punished drinking in rats have been studied in a number of laboratories using versions of the technique developed by Vogel and coworkers. There are several reports that buspirone can increase rates of punished drinking with more consistent effects apparently being seen after oral than after intraperitoneal or subcutaneous administration (Oakley and Jones, 1983; Taylor et al., 1985; Merlo Pich and Samanin, 1986). It is clear, however, that buspirone is not always found to be active in procedures sensitive to the effects of other anxiolytics and frequently shows an action much smaller in magnitude than that seen with benzodiazepine receptor ligands (Sanger et al., 1985; Haskins et al., 1989; Weissman et al., 1984; McCloskey et al., 1987). The significance of this activity in relation to buspirone's clinical action is unclear, and a report by Gower and Tricklebank (1988) has further complicated the interpretation of such findings. These researchers reported that punished drinking was facilitated in rats not only by buspirone, but also by its metabolite 1-(2-pyrimidyl) piperazine (1-PP), which is known to have α_2-adrenoceptor antagonist activity (Giral et al.,1987). Similar effects were seen with other α_2-adrenoceptor antagonists, including idazoxan and yohimbine. This is a very surprising result, since yohimbine has been reported to induce anxiety or arousal (Charney et al., 1982; Holmberg and Gershon, 1961). Furthermore, the α_2-adrenoceptor agonist, clonidine, which had no effect on punished drinking in the study of Gower and Tricklebank, has been found by other workers to produce an anxiolytic-like effect in a similar test, an effect that was antagonized by idazoxan (Soderpalm and Engel, 1988).

3.3. Punished Operant Responding

The most traditional conflict tests used in the analysis of anxiolytic drug action are punishment procedures using operant behavior. Typically, experimental animals are trained to emit a response, such as a lever press, that results in the delivery of food or water reinforcement according to the schedule in operation (Sanger and Blackman, 1989). A stable baseline of this behavior is established, generally by testing the animals several times each week over a period of a number of weeks. Then, a stimulus, such as the illumination of a lamp or the delivery of a noise, is presented at intervals. During this stimulus, the animals are able to obtain the reinforcers if they continue responding, but in addition to the schedule of positive reinforcement, a punishment procedure is also in operation. Thus, if they continue to respond, the animals will not only receive food, but also punishment, usually mild electric shock. If they stop responding, however, they receive neither food nor punishment.

In the first description of a fully automated version of such a punishment procedure used to study drug effects, Geller and Seifter (1960) presented data using rats trained to lever press for sweetened condensed milk on a variable-interval schedule, so that lever presses were reinforced every 2 min on average (VI 2 min). Every 15 min, a tone was presented for 3 min. During the periods of tone presentation, the schedule changed, and every lever press produced both milk delivery and electric footshock. This had the effect of suppressing responding during the tone with the degree of suppression dependent upon the level of shock.

Geller and his colleagues carried out a number of pharmacological experiments, and found that drugs from the benzodiazepine, barbiturate, and carbamate classes, known to have clinical antianxiety activity, could greatly increase rates of lever pressing during the punishment period. In contrast, a variety of other drugs, including chlorpromazine, morphine, and amphetamine, did not produce this effect (Geller, 1964; Geller and Seifter, 1962; Geller et al., 1962). Similar results have been reproduced in many laboratories, and there is a good correspondence between the drugs and doses active in such tests and those that exert clinical anxiolytic activity (Cook and Davidson, 1973).

Punished operant responding, therefore, remains an important experimental tool for analyzing anxiolytic drug effects. However, it is not generally considered suitable as a screening technique. The training of experimental animals, whether rats or other species, is very time-consuming, and it may require several weeks to establish a complete dose–response function for even a single compound. Furthermore, in order to maintain such baselines, shock levels need to be titrated carefully in individual animals. However, these procedures offer the enormous advantage that, with stable behavioral baselines, it is possible to make relatively precise comparisons between different drugs and doses, and produce results that can be systematically replicated both within and between laboratories. In addition, it is possible to compare the effects of a drug on rates of punished as well as unpunished responding in the same animals during the same experimental session. This may be of particular importance in the search for drugs having anxiolytic activity without the sedative and muscle-relaxing actions sometimes considered to be undesirable side effects. In recent years, a number of compounds have been described that act as partial agonists or mixed agonist–antagonists at ω receptors in the central nervous system. In experiments using punishment procedures, such compounds have been reported to increase rates of responding suppressed by punishment at doses far below those that alter rates of unpunished responding (Bennett et al., 1987; William et al., 1989;

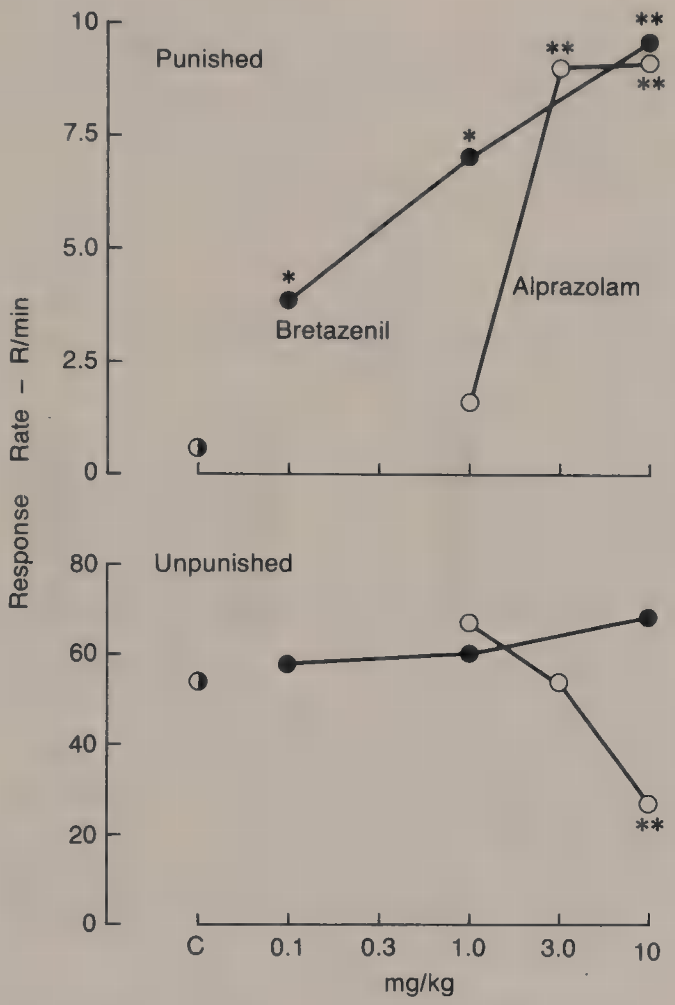

Fig. 2. The effects of the anxiolytic drug alprazolam and the ω (benzodiazepine) receptor agonist-antagonist bretazenil (Ro 16-6028) on punished and unpunished lever pressing in rats. The animals were trained to press a lever to obtain food pellets according to a VI 30-s schedule. At intervals, a white noise stimulus was presented, which indicated that responding would continue to be reinforced, but would also be punished. Dose-related increases in the low rates of punished responding were produced by both alprazolam and bretazenil. Alprazolam also decreased rates of unpunished responding at the highest dose, whereas bretazenil had no effect on this measure; $^{**}p < 0.01$ difference from control values.

Sanger et al., 1985; Martin et al., 1988). Such results are illustrated in Fig. 2.

As with other punishment procedures, the significance of operant methods for assessing anxiolytic activity has been called into question by recent results with buspirone and other related

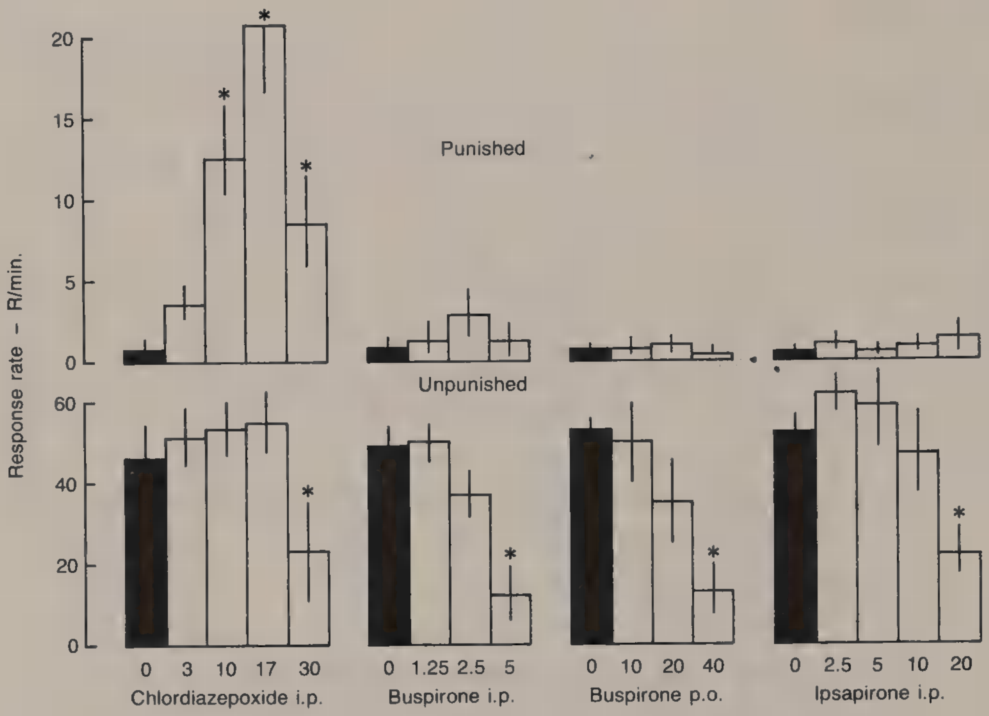

Fig. 3. The effects of chlordiazepoxide, buspirone, and ipsapirone on rates of punished and unpunished operant responding in rats. The same procedure was used as that described in the legend to Fig. 2. Chlordiazepoxide greatly increased rates of punished responding. Neither buspirone nor ipsapirone produced a similar effect; $^*p< 0.05$ difference from control values. Taken from Sanger (1990).

compounds. There have been several reports of the effects of buspirone on operant responding suppressed by punishment in rats and monkeys that have shown either that this drug was inactive or that it produced increases in punished responding considerably smaller than those seen with benzodiazepines and other anxiolytics (Geller and Hartmann, 1982; Young et al., 1987; Wettstein, 1988; Witkin and Perez, 1990; Howard and Pollard, 1990). Figure 3 shows that, in conditions where chlordiazepoxide greatly increased rates of VI reponding in rats suppressed by shock punishment, neither buspirone nor ipsapirone produced a similar effect.

Although punished behavior in rats and monkeys does not seem to be markedly affected by buspirone, quite different results have been observed with pigeons. In an important series of experiments, Barrett and coworkers trained pigeons to peck a key to obtain grain on a multiple fixed ratio (FR) schedule. When the key was illuminated in one color, the birds received grain after every 30th peck. When the color of the key changed, responses continued to be reinforced according to a similar FR 30 schedule, but each reinforcement was accompanied by electric shock punishment. Rates of punished responding were considerably lower than unpunished rates, and were substantially increased by administration of buspirone, ipsapirone, gepirone, 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin], and chlordiazepoxide. The results of sophisticated neurochemical experiments carried out in parallel with the behavioral manipulations indicated that the behavioral effects of all these compounds, with the exception of chlordiazepoxide, were mediated by reduced serotonergic transmission in the brain produced by activity at 5-HT_{1A} receptor sites (Barrett et al., 1986; Gleeson et al., 1989; Mansbach et al., 1988; Witkin and Barrett, 1986; Witkin et al., 1987).

The apparent difference between the effects of buspirone on punished responding in rats and monkeys on the one hand and pigeons on the other remains unexplained. Although mammalian species would normally be thought to provide a closer parallel with drug action in humans, these results suggest that, with anxiolytics, punished responding in pigeons might provide a better prediction of clinical activity, at least with buspirone and related compounds.This seems particularly paradoxical since other experiments point to similarities in the mechanisms of action of such drugs in different species. As noted above, Barrett's results show that the increased punished responding produced by buspirone in pigeons is probably associated with activity at 5-HT_{1A} receptors. Some behavioral effects of buspirone in rats are also mediated by these receptors. For example, in rats trained to discriminate between the 5-HT_{1A} agonist, 8-OH-DPAT, and saline, stimulus control generalizes to buspirone, ipsapirone, and related compounds, indicating an interoceptive stimulus produced by action at 5-HT_{1A} receptors (Tricklebank et al., 1987; Cunningham et al., 1987). In pigeons, there is also a cross-generalization between buspirone and 8-OH-DPAT, suggesting a similar mechanism (Mansbach and Barrett, 1987; Nader et al., 1989).

3.4. Passive Avoidance

Passive avoidance techniques have been very frequently used in psychopharmacology. They are relatively rapid and can induce marked changes in the behavior of experimental animals that may be sensitive to the effects of psychotropic drugs. In a typical experiment, a rat or mouse makes a simple exploratory response, such as stepping down from a raised platform or stepping through from one compartment to another in an exploration box, and receives an aversive electric shock. When the animal is retested some time later, usually 24 or 48 h, it typically does not step down or step through. Thus, the animal is apparently avoiding the aversive stimulation by withholding the response (hence passive avoidance). Although such methods are rarely described as punishment procedures, they can be considered as such because behavior is effectively suppressed by the application of shock contingent on the behavior.

Passive avoidance procedures have been most frequently used as a method for evaluating the effects of drugs on learning and memory. Drug administration is thus carried out before the first (acquisition) trial or immediately after this trial, and animals are subsequently tested without the drug (retention trial) to assess whether learning has been disturbed or enhanced by the drug. A number of studies have shown that, when benzodiazepines are administered before the acquisition trial, animals generally behave during the retention trial as if they had not been shocked on the first trial (Soubrié et al., 1976; Oishi et al., 1972). Thus, these drugs disrupt the acquisition of a passive avoidance response, an effect that is usually discussed in terms of drug action on learning, although other interpretations are possible. Such actions may parallel the disturbances of memory function produced by these drugs in humans (Curran, 1986; Lister, 1985).

If anxiolytic benzodiazepines are injected before the retention trial, however, they generally do not change the behavior of experimental animals, which continue to withhold the response (Sanger and Joly, 1985; Stein and Berger, 1969; Oishi et al., 1972). This result seems somewhat paradoxical since, under these conditions, benzodiazepines do not interfere with response suppres-

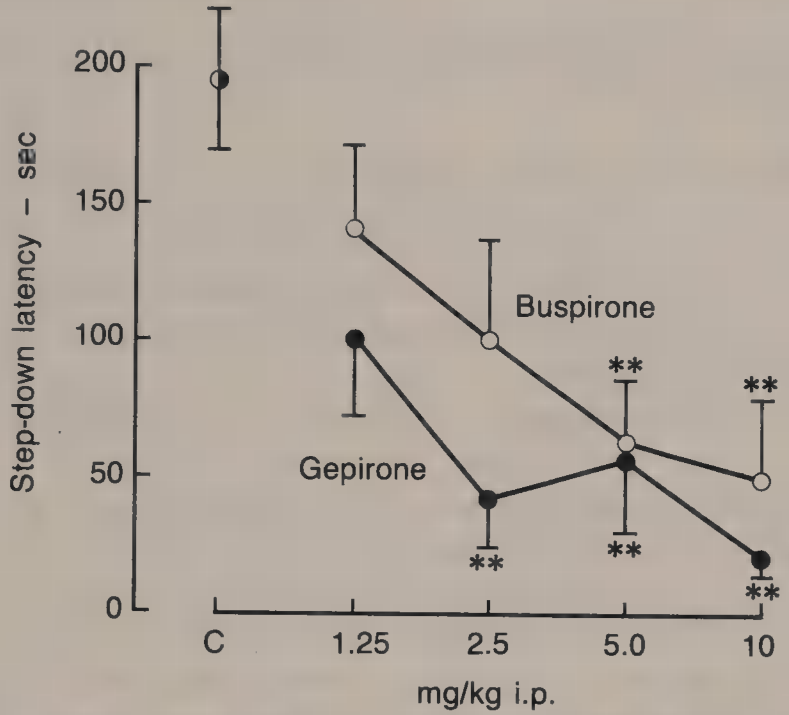

Fig. 4. Dose-related effects of buspirone and gepirone on the performance of a step-down passive avoidance response in rats. Control animals previously trained to remain on a raised platform showed long latencies to step off during the test. Treatment with buspirone or gepirone before the test produced dose-related decreases in these latencies; $^{**}p < 0.01$ difference from control animals. Taken from Sanger et al. (1989).

sion produced by punishment, in contrast to the effects of these drug in other punishment procedures.

Several recent reports, however, have shown that buspirone is effective in a passive avoidance procedure. If buspirone, or its structural analogs, ipsapirone and gepirone, are administered to rats trained in a passive avoidance procedure, the animals will emit the response as if they had not previously been trained to withhold it (Cullen et al., 1988; Sanger et al., 1989; Traber et al., 1984). This disinhibitory effect is illustrated for buspirone and gepirone in Fig. 4. This effect seems relatively specific to buspirone-like compounds. It was produced by 8-OH-DPAT and other compounds known to have agonist activity at the 5-HT$_{1A}$ subtype of the serotonin receptor, but was not shown by acute administration of haloperidol or acute or repeated administration of benzodiazepines or imipramine. It seems difficult, therefore, to describe such an action as an anxiolytic effect and to know whether it is of significance for the clinical activity of buspirone (Sanger and Joly, 1990).

4. Respondent Conditioning with Aversive Stimuli

The punishment procedures described in the preceding section, which have been so successful in evaluating and screening such anxiolytic drugs as benzodiazepines, involve the explicit association of the behavior of an animal with an aversive stimulus. In a sense, the presentation of aversive stimuli is under the control of the animal because, if the animal ceases to emit the punished response, it is able to avoid the aversive stimulus. Such considerations make it difficult to conceptualize the behavioral control exerted by punishment procedures in terms of anxiety. Punishment techniques are often referred to as conflict procedures on the assumption that the animal's behavior is under the control of competing drives: for example, to approach and respond on the manipulandum to obtain reinforcement and to avoid responding in order not to experience the aversive stimulus. However, it has often been noted that animals do not appear particularly emotional when well trained on punishment procedures, and the type of behavior seen under such conditions seems essentially appropriate and adaptive rather than inappropriate as is the case with pathological anxiety. Many other writers have pointed out that punishment procedures have low face validity as models of clinical anxiety (Morgan, 1968; Millenson and Leslie, 1974; Houser, 1978). There are, however, a variety of other behavioral procedures involving behavioral changes produced by aversive stimuli or by previously neutral stimuli associated with aversive events that have been used for assessing the effects of antianxiety drugs. In some cases, these techniques have higher face validity, and perhaps construct validity (Gray, 1978) than punishment procedures. Detailed reviews of results obtained with such methods have appeared elsewhere (Houser, 1978; Gray, 1977). The present section will therefore describe several procedures for which novel results have been recently reported.

4.1. Conditioned Suppression

A method that has certain similarities with punishment procedures, but with at least one fundamental difference is that described as conditioned suppression or the conditioned emotional response (CER). Blackman (1977) has written a useful review of this technique. This method involves the pairing of a neutral stimulus, such as a light or noise, with an aversive stimulus, usually a brief, but unavoidable electric shock. After a number of pairings, which thus involve a process of respondent conditioning, the previously neutral stimulus itself induces behavioral changes. Usually the stimulus is presented to animals responding on an operant schedule for food or water reinforcement, and responding is found to be depressed in rate or completely suppressed during the stimulus. The most frequently used method is that first described by Estes and Skinner (1941), in which the stimulus-shock pairings are presented during regular sessions in which subjects work for positive reinforcement (on the baseline). Alternatively, the respondent conditioning can be carried out in a different environment, and only the stimulus (CS) can be presented during regular operant sessions (off the baseline conditioning).

The essential difference between conditioned suppression and punishment procedures is, of course, that the aversive stimuli are unavoidable during conditioned suppression. Thus, even if the animal stops responding completely during the shock-associated stimulus, this will have no influence on whether the shock is presented. (There has, however, been discussion as to whether the animal is in fact able to modify the aversiveness of the stimulus by suppressing operant responding and what, exactly, the nature of this response suppression is; Blackman, 1977.) It is thus possible to argue that conditioned suppression has considerable face validity as a test of anxiety, because the animal is behaving maladaptively by ceasing to respond during the CS, since this leads to a reduction in positive reinforcers obtained with no change in the presentation of aversive stimuli.

Certainly, the apparent attractiveness of this method as a means of assessing drug effects on a behavioral baseline modified by an anxiety-inducing or emotional stimulus led to considerable early psychopharmacological research. Several studies reported that reserpine and chlorpromazine, at that time referred to as tranquilizing drugs, could alleviate the degree of response suppression, particularly after repeated administration (Brady, 1956; Ray, 1964; Appel, 1963; Kinnard et al., 1962). Similar results have been described with morphine (Hill et al., 1957; 1967). However, other researchers have failed to obtain comparable effects with these same drugs (Yamahiro et al., 1961; Lauener, 1963; Sanger, 1990).

There were several reports that benzodiazepines and barbiturates attenuated conditioned suppression, although meprobamate apparently exerted less clear effects (Lauener, 1963; Miczek, 1973; White and Holtzman, 1983; Ray, 1964). However, previous reviews of drug action on conditioned suppression have pointed out that, even with this class of drug, results have been much less consistent than with punishment procedures, even taking into account that a variety of different procedures were used in early studies and that a number of factors made interpretation of conditioned suppression particularly difficult (Gray, 1977; Houser, 1978; Millenson and Leslie, 1974).

Given the sensitivity of punishment procedures to benzodiazepines and barbiturates, conditioned suppression was clearly not a procedure of choice for evaluating anxiolytic drug action, and thus, there have been relatively few studies published using this method in recent years. Several studies also set out to make direct comparisons of drug effects on response suppression produced by contingent and noncontingent shocks (McMillan and Leander, 1975; Hymowitz and Abramson, 1983; Huppert and Iversen, 1975; Rawlins et al., 1980). In most cases, benzodiazepines were reported to produce much clearer attenuation of response suppression produced by punishment than of suppression associated with noncontingent shocks.

In contrast to the variable effects of buspirone and related compounds on the suppression of operant responding in rats by punishment, one recent study found that buspirone was effective in alleviating conditioned suppression in this species (Sanger, 1990). Figure 5 shows results obtained in this experiment with

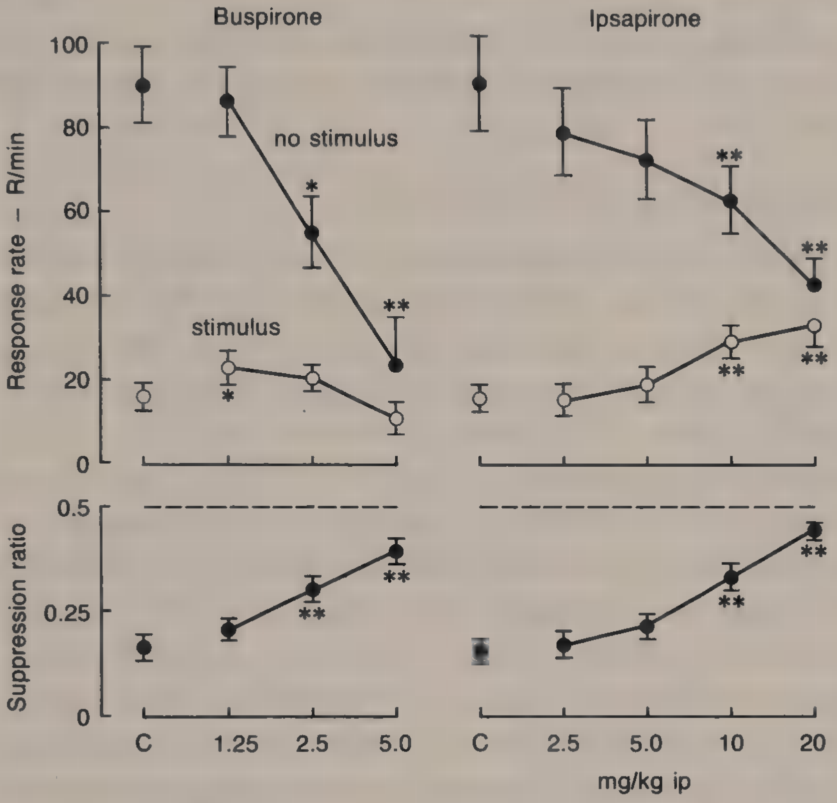

Fig. 5. The effects of buspirone and ipsapirone on the operant responding of rats during a conditioned emotional response procedure. Rats were trained to press a lever to obtain food according to a VI 30-s schedule, and from time to time, a visual stimulus was presented that terminated with a brief, unavoidable shock. Response rates were greatly reduced during the stimulus. Buspirone and ipsapirone decreased rates of unsuppressed responding but, at the same doses, also increased rates of suppressed responding. The suppression ratios were calculated as the ratios of unsuppressed and suppressed response rates, and could take values between 0, indicating complete response suppression during the preshock stimulus, and 0.5, indicating that response rate was unchanged during the stimulus; *$p < 0.05$, **$p < 0.01$ difference from control values. Modified from Sanger, 1990.

buspirone and ipsapirone. Both of these compounds decreased rates of unpunished responding, but also, at some doses, increased punished response rates giving rise to dose-related decreases in the degree of response suppression. Similar effects were observed with benzodiazepines, but not with drugs from several other classes. These results indicate that conditioned suppression may provide a procedure more sensitive than punished responding for assessing the activity of buspirone-like anxiolytics. It will be necessary, however, for these findings to be extended and replicated in other laboratories before this conclusion can be drawn with any certainty.

4.2. Potentiated Startle Response

Another procedure that involves respondent conditioning and the subsequent modification of behavior by a conditioned stimulus is the potentiated startle response. This technique is of particular interest because Davis and his colleagues have made an extensive study of the effects of various lesions and different pharmacological agents on this behavior. Many anatomical and pharmacological data are available, and thus, any researcher wishing to make use of this method for screening or evaluating novel compounds will find a body of comparative results in the literature (Davis, 1990).

The experimental procedure has two parts. First, subjects are presented with a number of pairings between a neutral stimulus (usually a light) and an aversive stimulus (usually an electric shock). This protocol is assumed to be a form of fear conditioning, the light becoming associated with the shock and itself coming to induce an emotional state of anticipatory fear. No behavioral measures are taken during the conditioning session. The second part of the experiment involves the presentation of intense acoustic stimuli (tones) and the measurement of the subsequent whole-body startle responses using automated equipment. The basic phenomenon is that tones preceded by the conditioned stimulus produce startle responses with greater force than tones that are presented alone. This is the potentiation of the startle response first studied by Brown et al. (1951).

The explanation for the potentiation is believed to be the induction of fear by the stimulus, and therefore, drugs or other manipulations that selectively decrease the potentiated startle (i.e., without affecting normal, nonpotentiated, or baseline acoustic startle) may be considered to be affecting this emotional state (Davis, 1986).

According to Davis (1986), the advantages of this method can be listed as follows:

1. It is a relatively simple conditioning procedure involving the modification of a behavior (startle response) that is under the control of well-studied neural circuits.
2. The potentiated startle is defined as the within-subject difference between the amplitudes of startle responses produced on noise-alone and on CS-noise trials. This reduces the problem of between-subject variability, but emphasizes that comparisons between potentiated and nonpotentiated startle responses are essential. It might be noted here that not all pharmacological studies with this technique have made such comparisons, and indeed, some workers have found that between-animal designs produce more satisfactory results (Hijzen and Slangen, 1989).
3. Different levels of noise can be used within a session to elicit different degrees of nonpotentiated startle. In this way, it should be possible to titrate levels of nonpotentiated and potentiated startle, so that the effect of a drug can be assessed on similar levels of each. This is an important consideration, because it should make drug effects much easier to interprete without problems of baseline dependence (Dews and Wenger, 1977; Sanger and Blackman, 1976). In practice, such manipulations seem rarely to be employed.
4. Shocks are not presented during testing sessions, thus eliminating the possibility that drugs might modify behavior because of analgesic activity.
5. The technique does not involve any obvious operant response, and thus, drug effects are not complicated by the possibility of effects on such behavior.

These are important arguments, and indeed, the potentiated startle response has been used successfully for analyzing drug action. For example, Davis (1979) described the effects of several doses of diazepam on potentiated and nonpotentiated startle. Diazepam produced a dose-related decrease in potentiated startle without affecting nonpotentiated startle. The interpretation of this result was that the drug reduced the effectiveness of the light stimulus to increase the amplitude of the startle response, because it decreased the state of fear induced by the stimulus.

In subsequent experiments, a variety of other compounds were reported to produce similar effects. Potentiated startle can be selectively blocked not only by the classical anxiolytic drugs diazepam and sodium amytal (Davis, 1979; Chi, 1965), but also by alcohol, morphine, nicotine, propranolol, and clonidine. Of particular interest is the fact that recent results have described similar effects for buspirone (Kehne et al., 1988; Mansbach and Geyer, 1988). Kehne et al. (1988) reported that, at doses of 2.5 and 5.0 mg/kg given by the subcutaneous route, buspirone decreased potentiated startle without affecting startle produced on the noise-alone trials. Thus, in this procedure, benzodiazepines and buspirone produce similar effects on the potentiated startle, although not on the nonpotentiated startle, which tends to be increased by buspirone and related compounds. This is clearly an interesting result, although the activity of several other drugs whose status as anxiolytics is disputable (clonidine, morphine, nicotine, and propranolol) may be considered a cause for concern (Davis, 1990).

The apparent similarity of the effects of diazepam and buspirone on potentiated startle seems to encourage the view that this technique is a valid one for evaluating the activity of clinically active anxiolytics. However, more recent studies have not always been able to demonstrate a clear distinction between the doses of diazepam that depress potentiated startle and those that depress nonpotentiated startle (Berg and Davis, 1984). It is also worth pointing out that the doses of buspirone found to be active are relatively high, showing depressant behavioral activity in a variety of other test procedures, and are certainly much higher than those reported to have activity associated with anxiolytic compounds in other procedures (e.g., Soderpalm et al., 1989). Furthermore, it is disappointing to note that studies of other compounds that, like buspirone, have affinity for 5-HT_{1A} receptors carried out in two different laboratories have reported differing results. Davis and coworkers (Kehne et al., 1988, Davis et al., 1988) found that buspirone and gepirone depressed potentiated startle, but that ipsapirone was active only at a relatively high dose and that 8-OH-DPAT was inactive. On the basis of these and other results, these researchers concluded that the activity of buspirone did not involve 5-HT mechanisms and that such mechanisms did not play an important role in mediating the potentiated startle response. In contrast, Mansbach and Geyer (1988) found similar activity with buspirone, gepirone, ipsapirone, and 8-OH-DPAT. Although such discrepancies are presumably the result of procedural differences that require further analysis, they do not aid the evaluation of the reliability and validity of the technique. Nevertheless, the potentiated startle response does seem to have a role to play in the analysis of anxiolytic drug action and, potentially, in the screening of novel compounds. In this respect, it is interesting that Glenn and Green (1989) recently reported that the 5-HT_3 antagonist, odansetron (GR 38032F), also depressed the potentiated startle response. This may thus be one the few behavioral procedures in which benzodiazepines, buspirone, and other 5-HT_{1A} agonists, and a 5-HT_3 antagonist have been reported to exert similar activity.

5. Exploratory Behavior

Tests of locomotor activity and exploration in rodents have been widely used for assessing the effects of psychotropic agents. Many ingenious varieties of apparatus have been employed, including Y-shaped mazes (Marriott and Spencer, 1965), open fields (Archer, 1973), hole boards (Nolan and Parks, 1973), Berlyne, boxes (Robbins and Iversen, 1973), and two-compartment boxes (Crawley and Goodwin, 1980). The advantages of such tests are that they are usually rapid, and do not require complex and expensive equipment, but can often be automated. Their disadvantage is that the behavioral significance of the different activities observed and quantified is not always clear, and thus, the interpretation of drug action is sometimes difficult (Robbins, 1977).

Studies of the effects of anxiolytic drugs on behavior in novel environments are of interest because of the hypothesis that exploratory behavior is under the control of two competing drives. It has been proposed by a number of authors (Montgomery, 1955; Kelley et al., 1989) that rodents are driven to approach and investigate novel objects and places, but that novelty also induces fear, which would tend to suppress exploration or produce active avoidance of the novel situation. Observed exploratory behavior is thus the result of these two competing drives and will depend to a large extent on the degree of novelty of the situation, as well as on other aspects of the animal's past experience (e.g., whether or not the animal has been deprived of food or water).

Clearly, drugs (or other manipulations) that reduce the presumed inhibitory mechanism would be expected to increase levels of exploration, unless they also have inhibitory effects on responses to novelty (Gray, 1977). As noted above, many different tests have been used for studying drug action, and it would not be useful to review all the studies that have involved anxiolytic and potentially anxiolytic drugs particularly since other reviews are available (Crawley, 1985; File, 1985b). Accordingly, in this section, several procedures will be described that have been proposed as measures of responses to novelty that are particularly sensitive to anxiolytic drug action.

5.1. Staircase Test

The staircase test is a relatively rapid and simple procedure involving easily quantified aspects of behavior. A rat or mouse is placed in a test box containing a five-step staircase. During a 3-min test, the activity of the animal is assessed as the number of steps climbed (both ascents and descents) and the number of times the animal rears on its hind paws. In the first descriptions of this procedure (Thiebot et al., 1973, 1976), it was proposed that rearing was a sign of anxiety and, thus, would be decreased by anxiolytic drugs, which might also increase the number of steps climbed. An alternative formulation (Steru et al., 1987) is that increased numbers of steps climbed indicate anxiolytic activity, whereas decreases in rearing provide a measure of sedation. The procedure has apparently not been subect to many procedural or behavioral manipulations in order to attempt its validation as a measure of fear or anxiety. However, the relatively few publications available describing experiments with this method have studied a large number of drugs, and thus, a pharmacological validation should be possible. Thiebot and her colleagues (1973,1976) found that several benzodiazepines, pentobarbital, and some other compounds increased the number of steps climbed without, in general, increasing the number of rears and, at higher doses, decreased rearing. Chlordiazepoxide and lorazepam decreased rearing without increasing the number of steps climbed, and several nonanxiolytics, including thioridazine, diphenhydramine, imipramine, and propranolol, decreased both steps climbed and rears at similar doses.

Several more recent studies have adapted the staircase test for use with mice and have produced similar results. Simiand and coworkers (1984) found that chlordiazepoxide, diazepam, clorazepate, phenobarbital, meprobamate, and valproate increased the numbers of steps climbed and decreased rearing, although with the benzodiazepines the increase in steps was not statistically significant. This latter observation is in contrast to a more recent study using a different strain of mice (Steru et al., 1987). Once again, lorazepam did not increase steps climbed, but rearing was decreased to a greater extent than steps climbed. Several other compounds, including neuroleptics and antidepressants, produced identical decreases in both measures.

On the basis of these results, Simiand and colleagues concluded that the staircase test was selective for anxiolytic drugs and was a suitable screening test for such compounds. In striking contrast, Pollard and Howard (1986) used the same method and obtained results that were, in general, similar, but drew the conclusion that the test lacked specificity and thus had limited use in screening. These authors reported that, although chlordiazepoxide, ethanol, and meprobamate were active, alprazolam was not (but Steru and coworkers found alprazolam to be active), nor was buspirone, thus providing at least two false negatives. Morphine also seemed to be a false positive. Figure 6 shows the results of a study using the staircase test with rats in which buspirone produced only decreases in both measures, which is consistent with the findings of Pollard and Howard (1986).

One particularly interesting observation made by Pollard and Howard was that CGS 9896, a compound known to have partial agonist or mixed agonist/antagonist activity at benzodiazepine binding sites (Bennett and Petrack, 1984), increased steps

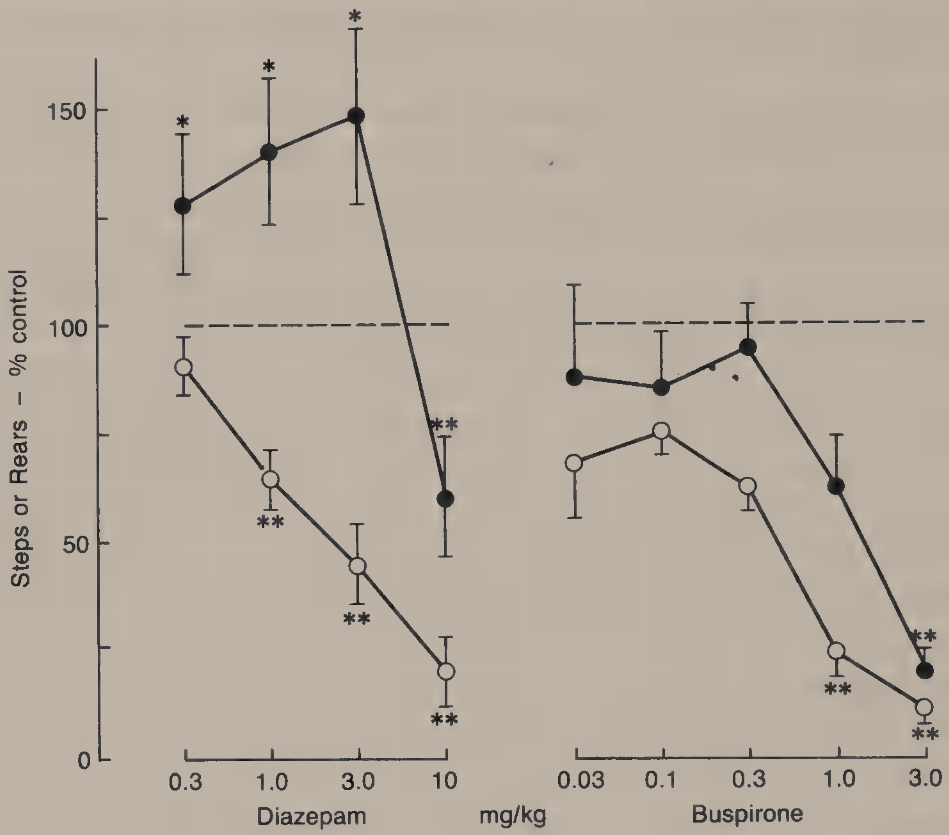

Fig. 6. The effects of diazepam and buspirone on the behavior of rats in the staircase test. Diazepam increased the number of steps climbed (●) at lower doses and decreased this measure at the highest dose. At the same time, rearing (○) showed a dose-related decrease. Buspirone produced only decreases in both measures. $^{*}p < 0.05$; $^{**}p < 0.01$ difference from control animals.

climbed at three doses and produced a smaller increase in rears at one dose. The authors concluded that this indicated a lack of anxiolytic activity, which could not be adequately assessed since no reports of clinical anxiolytic activity for this compound were available. However, CGS 9896 does have activity in other tests, increasing rates of punished responding, for example (Bennett and Petrack, 1984; Sanger et al., 1985). Belzung and coworkers (Belzung et al., 1989) recently reported that bretazenil (Ro 16-6028), another compound reported to have partial agonist or mixed agonist–antagonist activity at ω receptors (Potier et al., 1988; Sanger, 1987a), had no effect on either steps mounted or rears in mice. Similar doses were active in altering the behavior of mice in other situations, however, and other studies have reported anxiolytic-like activity of bretazenil in rodents (Martin et al., 1988).

The assessment of such effects in the staircase test will have to wait until the results of clinical trials with compounds, such as CGS 9896 and bretazenil, are published. However, data currently available seem to indicate that the original proposal (Thiebot et al., 1973,1976) that decreased rearing indicates anxiolytic activity is not correct and that Steru et al.'s (1987) reformulation may be more accurate. As a method for detecting anxiolytic activity of benzodiazepine and barbiturate-like compounds, it seems to be as sensitive and selective as many other tests. However, buspirone produces a different profile of activity, and the differences between results obtained in different laboratories would seem to be a cause for concern. Further research with this technique might usefully analyze the behavior observed in greater detail and perhaps carry out manipulations aimed at clarifying the relationship of the behavior to states of fear or anxiety.

5.2. Elevated Mazes

Another test of exploration that has become popular in recent years as a measure of anxiolytic drug action is the behavior of rats or mice in an elevated maze. Early work by Montgomery (1955) and others showed that rats tended to avoid open arms of mazes, which was taken as an indication of fear (presumably of height or open spaces). Morrison and Stephenson (1970) argued that fear- or anxiety-decreasing drugs should have increased the frequency and duration of entries into the open arm of a Y-shaped maze, and found that chlordiazepoxide, diazepam, pentobarbital, and amylobarbital exerted just such an effect, whereas a variety of drugs from other classes (amphetamine, nicotine, imipramine, atropine, and chlorpromazine) did not.

More recent studies have used elevated mazes in the shape of an X (Handley and Mithani, 1984) or + (elevated plus maze) (Pellow et al., 1985; Lister, 1987). The rat or mouse is placed in the center of the maze, and the number of entries and amount of time spent in the open and closed arms are recorded during a brief trial (usually 5 min). In a number of experiments, the exploratory behavior of the animals on a hole board was studied immediately before they were placed in the maze, but this did not seem to alter the pattern of maze exploration or the effects of different drugs (Pellow et al., 1985; Lister, 1987).

In contrast to the published studies of the staircase test described above, the plus maze has been subject to considerable analysis in attempts at its validation as a measure of fear or anxiety. Pellow and coworkers (1985) found similar levels of activity in Lister and Wistar rats, and observed that time spent in the open arms was associated with more immobility, freezing, defecation, and higher plasma corticosterone levels than time spent in the closed arms. In addition, activity in the maze did not change during several days of repeated testing. This latter finding was also reported by Lister (1987) working with mice and is interesting, since it suggests that novelty is not an important determinant of the behaviors displayed on the maze. Lister also carried out intercorrelations between several aspects of the behavior of control mice on the plus maze and the holeboard and concluded, on the basis of a factor analysis, that the behavior could be described in terms of three factors. The first factor, which was associated with the proportion of entries into and time in the open arms, was assumed to be an indication of fear or anxiety, whereas the two other factors were associated with directed exploration and locomotor activity (Lister, 1990).

In addition to these systematic studies of behavioral aspects of this procedure, researchers from several laboratories have described the effects of a number of pharmacological agents. In confirmation of the findings of Morrison and Stephenson (1970), using a Y maze, a number of studies have shown that chlordiazepoxide, diazepam, pentobarbital, and amylobarbital increase the proportion of entries into and time in the open arms, and similar effects have been reported for ethanol, alprazolam, CL 218,872, and tracazolate (Stephens et al., 1986; Handley and Mithani, 1984; Pellow et al., 1985; Pellow and File, 1986; File and Pellow, 1985; Lister, 1987; Moser, 1989). Similar effects have been reported less frequently or less consistently with clonidine and other α_2-adrenoceptor agonists (Handley and Mithani, 1984), metergoline (Pellow et al., 1987), pindolol, ritanserin, and related compounds (Critchley and Handley, 1987, but *see also* Pellow et al., 1987).

A number of compounds have also been reported to decrease the proportion of time in or entries into the open arms, which has been interpreted as an increase in fear of these arms or an anxiogenic effect. However, these results appear to show considerable differences in different laboratories. Thus, at least two reports of such presumed anxiogenic activity are available for picrotoxin, yohimbine, caffeine, and FG 7142 (Handley and Mithani, 1984; Pellow et al., 1985; Lister, 1987). Handley and Mithani (1984) also reported that, in addition to yohimbine, a variety of other compounds known to have α_2-adrenoceptor antagonist activity decreased entries into and time in the open arms. Several other research groups have been unable to confirm these results, however (Durcan et al., 1989; Moser, 1989).

Further complication is added by studies of buspirone and related compounds. Pellow and File (1986) reported that, instead of increasing the percentage of time in the open arms, buspirone, at a dose of 0.5 mg/kg, decreased this measure, and in another study (Pellow et al., 1987), higher doses produced a similar effect. This activity has been confirmed in other laboratories, and similar effects have been reported for the buspirone analog ipsapirone and for other compounds known to be ligands for the 5-HT_{1A} receptor (Pellow et al., 1987; Critchley and Handley, 1987; Moser, 1989). In contrast, two reports have described opposite (i.e., anxiolytic-effect) activity for these procedures (Soderpalm et al., 1989; Dunn et al., 1989). It is not clear why such strikingly different results should have been obtained in different laboratories. It might be pointed out, however, that Soderpalm et al. (1989) reported activity only at very limited ranges of low doses.

Elevated mazes have thus provided convenient methods for analyzing drug action, and have been subjected to considerable analysis as measures of fear or anxiety. Although the plus maze has found favor with some researchers, a number of inconsistencies and anomalous findings are to be found in the literature. It is not clear that the procedure has been adequately validated pharmacologically. As Moser (1989) points out on the basis of his findings with buspirone, such results indicate that the plus maze "...cannot be regarded as a model for human anxiety" and may not provide the best method for laboratory analysis of potentially anxiolytic compounds as yet untried in the clinic. It might be added that, since a number of compounds have been reported to have anxiogenic activity on the basis of their effects on plus maze exploration, but are not known to produce a state similar to pathological anxiety in humans (e.g., buspirone and mianserin), caution is necessary in interpreting the effects of drugs that decrease exploration of the open arms of the maze (*see also* Thiebot et al., 1988).

5.3. Social Interaction

Another procedure that may, for the sake of convenience, be classified as a method involving responses to novelty is the social interaction test as developed by File and coworkers. This method also assumes that novel aspects of a rat's environment generate anxiety leading to lower than normal levels of certain behaviors, which are consequently facilitated by administration of anxiolytic drugs. This test, however, adds a further level of complexity, since it involves the quantification of aspects of behavior in pairs of rats, both of which are treated with the same drug or vehicle.

Before describing this procedure, it is relevant to point out here that many studies of the effects of anxiolytic drugs on another social behavior, aggression, have been carried out. Indeed, the original development of chlordiazepoxide as a tranquilizer was based on the observation that it had a calming effect on aggressive monkeys. There have subsequently been a great many demonstrations that benzodiazepines can reduce aggression in primates and rodents, although many of these effects are now believed to result from muscle relaxant or sedative actions rather than a more specific antiaggression effect. The effects of these drugs on aggression are complex, and increases in aggression have also been reported. This area of research has been reviewed recently by Miczek and Winslow (1987), as has the more general area of the analysis of drug action on social interaction in rodents (Brain et al., 1989). The present section will therefore concentrate on the procedure described by File as a model of anxiety specifically developed for assessing anxiolytic drug action.

File (1980) set out to design a procedure that would not involve electric shock punishment or deprivation of food or water, and would be more relevant to human social anxiety. Pairs of rats, unfamiliar with each other, were placed in an arena, and their behavior assessed for a period of 10 min. The extent to which the arena was assumed to be fear-inducing was manipulated by changing the level of illumination and whether or not rats had previously been exposed, singly, to the arena. This method provided four test conditions—low light/familiar arena, high light/unfamiliar arena, low light/unfamiliar arena, and high light/familiar arena—which were assumed to vary in the extent to which they induced fear, anxiety, or uncertainty in the rats. The amount of time for which the animals were in social contact differed in these different conditions, with the smallest amount being observed in the high light/unfamiliar condition. Photocells were also used to quantify locomotion, and it was proposed that manipulations that increased social interaction without increasing locomotion might be doing so by reducing anxiety.

In pharmacological studies, it was found that, after acute administration of chlordiazepoxide, both social interaction and locomotion were decreased under all conditions. However, after five, once daily injections of this drug, it no longer decreased locomotion, and levels of social interaction were similar in all test conditions, thus showing considerable increases in the conditions assumed to be most anxiety-provoking (File and Hyde, 1978). Tolerance developed to this effect of chlordiazepoxide, however, after 15–25 daily administrations of the drug (Vellucci and File, 1979).

In studies with other drugs, diazepam and ethanol, like chlordiazepoxide, produced the greatest increases in social interaction in the unfamiliar conditions where control levels were lowest, whereas phenobarbital and meprobamate did not give rise to similar effects even with repeated administration. The benzodiazepines lorazepam and triazolam produced no increases in social interaction, whereas piracetam did (File, 1980; File and Hyde, 1979). Buspirone was reported to be inactive (File, 1985a).

These results do not seem to produce a very successful pharmacological validation of the social interaction test, because some anxiolytic drugs (e.g., lorazepam and buspirone) were inactive, but piracetam, which is not known to be anxiolytic or indeed to share any pharmacological properties with benzodiazepines, was active. Nevertheless, other researchers have made use of the social interaction test and apparently consider it a useful tool for assessing anxiolytic drug action.

Gardner and Guy (1984; Guy and Gardner, 1985) used a modified version of this procedure to investigate the effects of a variety of pharmacological agents. The studies described by these authors differ in many respects from those of File and coworkers, including the duration of the test, the route of drug administration, and the method of assessing locomotion, which was done by direct observation, rather than with photocells, and thus presumably was not confounded with aspects of social behavior. Perhaps the most significant difference, however, was the separation by Gardner and Guy of social contact into two behavioral categories, which they labeled social interaction and aggression. Levels of social interaction (not including aggression) in control animals were lower than those reported in previous studies, and were increased after acute, oral administration of a variety of benzodiazepines and by other compounds known to act as ligands at the same binding sites (CL 218,872, suriclone) as well as by sodium valproate. In many cases, measures of aggression were decreased by doses that increased social interaction, thus providing a potential explanation of why some of these results differed from those of previous studies. Several nonanxiolytics, including chlorpromazine, trazodone, and nortriptyline, were tested and found not to produce effects similar to those of the anxiolytics and, although the stimulant compounds amphetamine and caffeine increased social interaction, they also increased locomotor activity.

Another interesting aspect of the results reported by Guy and Gardner (1985) was their finding that oral administration of 5, 10, and 20 mg/kg of buspirone also increased social interaction without affecting aggression or, except at the highest dose, locomotor activity. These results were followed up by Dunn and colleagues (Dunn et al., 1989), who found that intraperitoneal administration of buspirone, gepirone, and 8-OH-DPAT increased time spent in social interaction and, in general, decreased locomotor activity. It is notable that the control levels of social interaction reported by these researchers (approx 100 s in a 300-s test) were considerably greater than the values reported by Guy and Gardner (approx 30 s in a 300-s test). Dunn et al. also reported that several compounds known to be competitive or noncompetitive antagonists of *N*-methyl-*d*-aspartate, including MK-801 and AP7, also produced small increases in the time spent in social interaction.

On the basis of this body of data, it is clearly difficult to draw definite conclusions concerning the validity of social interaction in rats as either a model of clinical anxiety or a suitable test for developing novel anxiolytic agents. Although some studies point to low validity, others suggest much greater specificity. It seems, however, that methodological details may be of major importance, because there are many differences between results reported from different laboratories. This point has again been emphasized recently by studies of compounds having antagonist properties at 5-HT_3 receptors using social interaction. Although some authors report anxiolytic-like activity for such compounds, others find them completely inactive (Jones et al., 1988; File and Johnston, 1989).

6. Discriminative Stimulus Properties

6.1. Stimulus Properties of Anxiolytics

Drug discrimination procedures have, in recent years, provided a methodology of major importance in psychopharmacology (Stolerman et al., 1989). In a typical experiment, animals are trained to make one response after injection of a drug and another response after a vehicle injection. Frequently, the responses are presses on two levers in an operant test chamber. With many psychotropic drugs, rodents and primates are able to learn such a discrimination, training usually requiring several weeks. The drug state of the animal may be considered to give rise to an interoceptive stimulus, which sets the occasion for a particular response. The experimental animals are thus considered to be discriminating between the internal stimuli produced by the drugged and nondrugged states (for reviews, *see* Colpaert, 1986; Jarbe, 1989).

Once animals have been trained to discriminate a particular drug, tests of substitution (generalization) and antagonism can be carried out with other pharmacological agents. Drug discrimination procedures are most frequently used in studies of drug classification and mechanistic investigations. They therefore differ from the other methods described in this chapter in which behaviors are generated in experimental animals as potential models of clinical disorders with the hope of demonstrating drug action predictive of clinical activity. However, since drug discrimination studies have been widely used with anxiolytic drugs, it seems appropriate to consider their significance as tools for analyzing the action of this class of agent (Andrews and Stephens, 1990).

Colpaert and coworkers (1976) demonstrated that, when rats were trained to discriminate chlordiazepoxide from saline, this stimulus control would generalize to other anxiolytic benzodiazepines and barbiturates. Rats trained with chlordiazepoxide responded on the lever associated with this benzodiazepine after administration of a number of drugs, including diazepam, lorazepam, oxazepam, pentobarbital, and phenobarbital. Nonanxiolytic agents, such as chlorpromazine and haloperidol, produced responding on the saline lever, however. Many subsequent studies have confirmed that benzodiazepines, other ω receptor ligands, barbiturates, and meprobamate could share discriminative stimulus properties (Garcha et al., 1985; Shannon and Herling, 1983a; Sanger, 1988), as illustrated in Fig. 7. There have been several contrary findings, however (Ator and Griffiths, 1989; Barry and Krimmer, 1979).

Of course, it is difficult to argue that the internal stimulus generated by an anxiolytic drug is related to its anxiolytic activity because, in most drug discrimination experiments, no explicit attempt is made to generate a state of fear or anxiety in the animals. Nevertheless, it is possible that the stimulus properties of these drugs are produced by pharmacological activity similar to

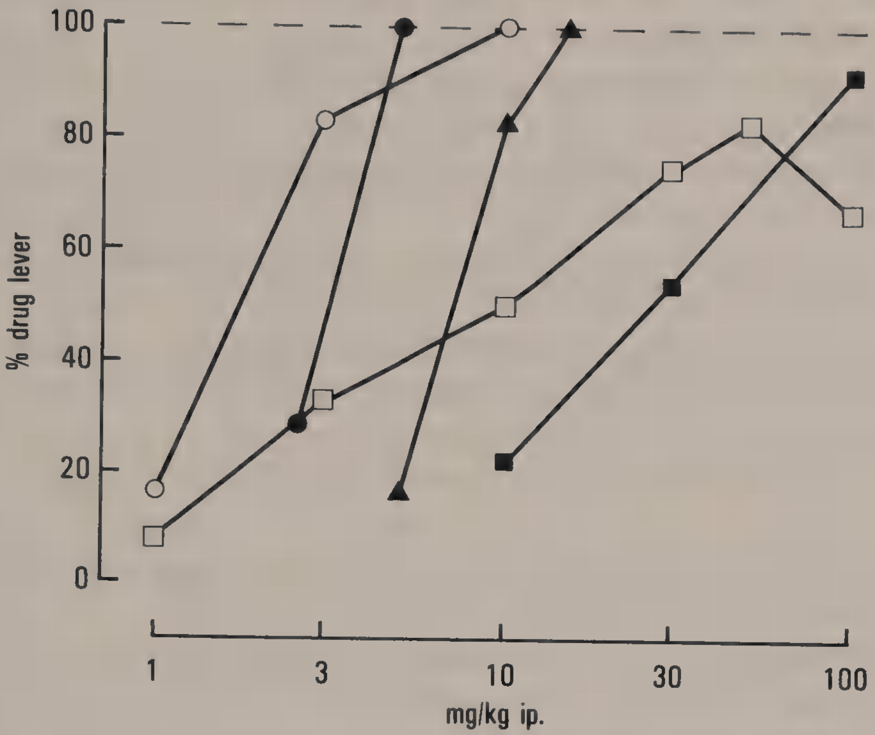

Fig. 7. The results of substitution (generalization) tests with a variety of compounds in rats trained to discriminate a dose of chlordiazepoxide (5 mg/kg) from saline. All drugs produced dose-related increases in the numbers of animals responding on the lever associated with the training drug. Modified from Sanger et al. (1985). ● Chlordiazepoxide; ○ zopiclone; ■ meprobamate; □ CGS 9896; ▲ pentobarbital. Modified from Sanger et al., 1985.

that involved in their antianxiety effects. Consistent with this possibility, several studies have shown that compounds that act as partial agonists at ω receptors and show antipunishment activity without sedation or muscle relaxation may share stimulus properties with full agonists (Ator and Griffiths, 1986; Sanger et al., 1985, 1987; Stephens et al., 1984; Bennett, 1985). In addition, differences have been reported between the discriminative stimulus properties of benzodiazepines and those of the imidazopyridine zolpidem, which although binding to ω receptors appears to exert selective hypnotic activity (Depoortere et al., 1986; Sanger and Zivkovic, 1986). On the basis of such results, it has been suggested that the discriminative stimulus properties of benzodiazepines may be related to anxiolytic activity, whereas those of zolpidem may be more closely associated with hypnotic effects (Sanger, 1988).

There are a number of findings inconsistent with this formulation, however. The partial agonist, CGS 9896, has been found in several studies not to be generalized by rats trained to discriminate benzodiazepines (Shannon and Herling, 1983b; Rauch and Stolerman, 1987), and such results have led to the proposal that the discriminative cue produced by diazepam may be associated with muscle-relaxant activity, whereas that produced by CGS 9896 itself may be associated with anxiolytic effects (Bennett, 1985, 1988). More recently, it has been reported that there are differences between the stimulus properties of benzodiazepines and those of the β-carboline, abecarnil (ZK 112119), and the imidazopyridine, alpidem, despite all these compounds having activity at ω receptors (Andrews and Stephens, 1989; Zivkovic et al., 1990). Since abecarnil is currently in clinical trials as an anxiolytic and alpidem has been shown to have clinical anxiolytic activity (Regnier et al., 1989), these results suggest that there is not a close association between anxiolytic action and discriminative stimulus properties of compounds active at ω receptors. Furthermore, it has been established quite clearly that cross-generalization does not occur between buspirone and related compounds, and benzodiazepines (Ator and Griffiths, 1986; Hendry et al., 1983; Rauch and Stolerman, 1987; Cunningham et al., 1987).

6.2. Discriminative Stimulus Properties of Pentylenetetrazol

Studies of the discriminative stimulus properties of anxiolytic drugs have considerable importance for characterizing the behavioral profiles and mechanisms of action of these agents. However, these techniques seem to have little relevance as animal models of the clinical state of anxiety. In contrast, another drug-induced discriminative stimulus, that produced by pentylenetetrazol, has been proposed as an animal model of anxiety (Lal and Emmett-Oglesby, 1983). Shearman and Lal (1979) found that it was possible to train rats to discriminate between saline and low doses of pentylenetetrazol, which at higher doses induces seizures. Pentylenetetrazol was apparently chosen for these studies, because it had been reported to induce anxiety in human volunteers (Rodin, 1958; Rodin and Calhoun, 1970). The stimulus properties of pentylenetetrazol were found to be antagonized by benzodiazepines, barbiturates, and meprobamate.

On the basis of such results, which have subsequently been confirmed in several laboratories (Stephens et al., 1987; Vellucci et al., 1988; Wilson and Bennett, 1989), it has been argued that pentylenetetrazol induces an internal (subjective) state in the experimental animals equivalent to human anxiety (Lal and Emmett-Oglesby, 1983; Lal and Fielding, 1984; Emmett-Oglesby et al., 1987). It is indeed the case that the stimulus properties of pentylenetetrazol are antagonized by a range of anxiolytic drugs, including some ω receptor partial agonists (Stephens et al., 1987; Spencer and Lal, 1983), and that several anticonvulsant drugs that are not known to be anxiolytic are inactive (Shearman and Lal, 1980). This suggests that the internal stimulus produced by pentylenetetrazol is more closely related to the anxiogenic rather than the convulsant properties of this compound (but *see* Andrews and Stephens, 1990). There does appear to be a close parallel between the compounds reported to antagonize the pentylenetetrazol discriminative stimulus and those producing antipunishment effects. Recent studies have shown that buspirone does not antagonize the effect of pentyleneterazol (Wilson and Bennett, 1989).

7. Overview and Conclusions

The purpose of the present chapter has been to describe a variety of behavioral tests used in the analysis of anxiolytic drug action and to make at least some assessment of their validity as models of anxiety. The review has necessarily been selective, and many procedures have not been described, including some that have recently been used with considerable effect, such as defensive burying (Treit et al., 1981), the exploration of a two-compartment box (Crawley and Goodwin, 1980; Costall et al., 1989), and ultrasonic vocalization (Gardner, 1985).

However, several of the most thoroughly studied methods have been described, and these techniques are representative of the majority of the studies carried out in this area of psychopharmacology.

It should be clear that most of the procedures described have some face validity as models of anxiety, although this may not stand up to much analysis. The term fear has been used in attempts to explain the behavior of experimental animals in many of the tests of punishment, respondent conditioning, and exploration. It is certain that fear and anxiety are related emotions, but they are not usually considered to be identical. Fear is generally thought to be adaptive, since it is usually produced by potentially harmful stimuli and may lead to appropriate responses, such as avoidance or escape. Pathological anxiety, however, is more likely to be inappropriate, because it is not produced by identifiable or evidently stressful stimuli, and thus, anxiety may lead to maladapted behavior. In the behavioral tests used to assess anxiolytic drug action, the behavior of the animals under control conditions (e.g., suppression of exploratory, consummatory, or operant responses) often appears adaptive. However, many anxiolytic drugs, and particularly barbiturates and benzodiazepines, have quite clear effects on these behaviors. This has led to the widespread use of many behavioral procedures with little discussion of their psychological validation, although their pharmacological validity has been relatively high.

The recent appearance of buspirone as a clinically effective anxiolytic, however, has forced laboratory researchers to reconsider the appropriateness of many behavioral tests. Buspirone and related drugs have behavioral actions that are often very different from those of benzodiazepines. In tests where benzodiazepines produce clear effects, such as punished operant responding in rodents and primates, buspirone often has little activity. In contrast, buspirone sometimes exerts effects, such as the disruption of a passive avoidance response, not shared by benzodiazepines. Reports that benzodiazepines and buspirone show similar activity, on the exploration of an elevated maze for example, are not always confirmed in different laboratories. Particularly confusing is the finding that buspirone produces much clearer increases in responding suppressed by punishment in pigeons than in other species. This result certainly deserves further analysis and, eventually, an explanation.

One of the most frustrating aspects of the psychopharmacology of anxiolytic drugs is the many reports of apparent differences between results obtained using similar techniques in different laboratories. Many examples of such divergent results have been described in the preceding sections. These differences are evidently produced by a multitude of, perhaps small, methodological differences that do not necessarily become clear even with the most detailed scrutiny of published reports. It is, of course, very clear that the behavioral effects of drugs may depend critically on experimental details, such as behavioral baselines or the past experience of experimental subjects (Barrett, 1984; Sanger, 1987b). The only method available to establish which are the important variables in determining the behavioral effects of anxiolytic and potentially anxiolytic drugs is to carry out further systematic, parametric studies. Such research currently seems to have a relatively low priority in many laboratories. However, it will probably need to be emphasized more firmly in the future if behavioral models are to be designed that are better adapted to the screening and development of novel, more effective anxiolytics.

One final comment is appropriate. There has been considerable recent progress in psychiatry in the description and classification of anxiety, and it is now considered that anxiety may take a variety of different forms, such as generalized anxiety, panic, and phobias. The present discussion has not been concerned with such distinctions, since they are only now being considered by researchers working on the development of animal models and the investigation of anxiolytic drugs. It is likely, however, that in the near future considerably greater efforts will be made to develop animal procedures more closely related to different clinical conditions (*see* chapter by Commissaris and Fontana, present volume). Such research may eventually provide animal models with greater psychological validity and may also resolve some of the problems of pharmacological validation outlined in the present discussion.

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Pharmacological Evaluation of Potential Animal Models for the Study of Antipanic and Panicogenic Treatment Effects

Randall L. Commissaris and David J. Fontana

1. Introduction

The present chapter reviews the effects of antipanic and panicogenic treatments in several animal models of anxiety, all of which have exhibited some degree of predictive validity based on positive effects following acute administration of benzodiazepine and barbiturate anxiolytic agents. In several animal models for anxiety (social interaction, elevated plus maze, and potentiated startle), three weeks of repeated daily treatment with tricyclic (TCA) or monoamine oxidase inhibitor (MAOI) antidepressants failed to exert anxiolytic-like effects. Moreover, only modest anxiolytic-like effects have been observed in the novelty-suppressed feeding (NS) and conditioned suppression of drinking (CSD) conflict tasks following three weeks of treatment with TCAs. However, when chronic treatment was extended to five weeks or longer, robust and significant anxiolytic-like effects were observed in the CSD paradigm following chronic treatment with several TCAs and nonselective MAOIs. Furthermore, the efficacy of atypical antidepressants and other agents to increase punished responding when administered chronically was correlated with the efficacy of these agents in the clinical management of panic disorder. Long-term chronic

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treatment (5–12 wk) with TCAs or MAOIs, however, did not alter defensive burying behavior. Thus, not all animal models for anxiety appear to be sensitive to the anxiolytic-like effects of antipanic treatments. Acute administration of various clinically effective panic-inducing treatments (yohimbine, sodium lactate, isoproterenol, *m*-chlorphenylpiperazine [mCPP] administration, or carbon dioxide exposure) yielded variable effects in these animal models. Thus, although none of the animal models for anxiety exhibited significant predictive validity when tested against panicogenic treatments, the CSD conflict paradigm and perhaps other paradigms as well (with a longer duration of chronic treatment) may be effective animal models for the study of the anxiolytic-like effects of antipanic treatments. Finally, it must be pointed out that the distinctions between the human conditions of panic disorder and generalized anxiety disorder (GAD), and the specificity (or lack thereof) of the drug treatments for these disorders are at the present time unresolved.

2. Clinical Characterization of Panic Disorder and Antipanic Treatments

2.1. Panic Disorder—Characteristics and Classification

Panic disorder is classified as a distinct anxiety neurosis (DSM-IIIR, 1987). Patients suffering from this disorder experience sudden, unprovoked, and extreme attacks of anxiety, bordering on terror; these panic attacks often are accompanied by such physical symptoms as shortness of breath, sweating, dizziness, and palpitations (DSM-IIIR, 1987). In addition, patients frequently report feeling that they are smothering, are going crazy, and are going to die. The primary defining feature of panic disorder is the presence of these discrete patient-reported panic attacks. In contrast to panic disorder, generalized anxiety disorder (GAD) is an anxiety neurosis characterized by generalized, persistent anxiety that is continuous for at least 1 mo, and has the symptoms of motor tension, autonomic hyperactivity, apprehensive expectation, and vigilance and scanning (DSM-IIIR, 1987).

2.2. Pharmacological Treatment of Panic Disorder

2.2.1. The Requirement for Chronic Treatment

Antipanic therapy is almost always chronic in nature, with prophylaxis of panic attacks being the primary therapeutic goal. There currently is no short-term (i.e., onset within minutes) antipanic treatment available for patients suffering from panic disorder. The latency to onset of antipanic effects with most clinically effective agents may be as long as 3–4 wk. Moreover, maximal antipanic efficacy often is not observed until at least 5 wk of chronic treatment (Klein and Rabkin, 1981; Sheehan et al., 1980; Sheehan, 1986; Charney et al., 1986; Mavissakalian et al., 1983, 1987).

2.2.2. Clinically Effective Agents

An interesting array of pharmacological agents has been found to exhibit antipanic effects in patients suffering from panic disorder. Many of these agents are thought of primarily as antidepressants, although not all antidepressant agents are antipanic agents. In contrast, antiGAD treatments, like traditional benzodiazepines and barbiturates, often are of limited utility in the treatment of panic disorder. The similarities and distinctions in the clinical efficacy of various agents to serve as antidepressant, antipanic, and/or antiGAD treatments are described below. Using this data base, various animal models for panic disorder will be evaluated from the perspective of their predictive validity.

2.2.2.1. ANTIPANIC VS ANTIGAD TREATMENTS

Both tricyclic antidepressants (TCAs) and nonselective monoamine oxidase inhibitors (MAOIs), when given chronically (>3 wk), are effective in reducing the frequency and/or severity of panic attacks in patients with panic disorder (Klein, 1964,1967; Garakani et al., 1984; Sheehan et al., 1980). In contrast, these agents have been reported to be less effective against the symptoms of GAD (Klein, 1964,1967,1980; Klein and Fink, 1962; Klein and Rabkin, 1981; Zitrin et al. 1980,1981,1983). It should be noted, however, that imipramine and other TCAs have been reported by some investigators to exert antiGAD effects (McNair and Kahn, 1981; Kahn et al., 1986; Lipman et al., 1986; Barlow, 1988). MAO exists as two isozymes, referred to as MAOA and MAOB; although chronic treatment with the nonselective MAOI phenelzine has been reported to exert antipanic effects (Sheehan et al., 1980), the potential antipanic efficacy of MAOA- or MAOB-selective inhibitors (clorgyline or deprenyl, respectively) has not been determined.

On the other hand, acute administration of traditional antianxiety agents, such as diazepam, chlordiazepoxide, or phenobarbital, effectively treat GAD, but these agents often have been regarded as less effective in preventing panic attacks (Klein, 1964,1980; Sheehan, 1982,1985,1987). Recently, however, the "second-generation" benzodiazepines, alprazolam and clonazepam, have been reported to be effective antipanic agents (Sheehan et al., 1984; Chouinard et al., 1982; Shehi and Patterson, 1984; Beaudry et al., 1986; Pollack et al., 1986; Spier et al., 1986). Moreover, lorazepam (Charney et al., 1987a; Rickels and Schweizer, 1986) and diazepam (Noyes et al., 1984) have been reported to exert at least modest antipanic effects. Findings by Cohn (1981), however, suggest that diazepam does not have the antipanic efficacy of alprazolam. The reason for this apparent difference in antipanic efficacy among benzodiazepine agents remains to be determined.

2.2.2.2. ANTIPANIC VS ANTIDEPRESSANT TREATMENTS

Antipanic efficacy cannot be correlated to antidepressant efficacy, since not all antidepressant agents are efficacious antipanic treatments. Indeed, there is a range of antipanic efficacy across atypical antidepressants. For example, the serotonin reuptake blocking antidepressants fluvoxamine and fluoxetine are highly efficacious antipanic agents (*see* review by Sheehan et al., 1988), whereas the novel antidepressant buproprion has been shown by Sheehan and coworkers (1983) to exhibit no antipanic effect. As an antidepressant without antipanic effects, buproprion represents a critically important negative control agent for evaluating a potential animal model for studying the neuropharmacology of antipanic treatment effects. Moreover, the atypical antidepressant trazodone has been shown to be an effective antipanic agent, but this agent does not appear to possess the antipanic efficacy seen with alprazolam or imipramine (Charney et al., 1986; Mavissakalian et al., 1987).

Unfortunately, the potential antipanic effects of several clinically effective antidepressant treatments have not been examined or reported. For example, electroconvulsive therapy (ECT) has not been tested rigorously as an antipanic treatment, although an early report by Klein (1964) suggests that ECT is not an effective antipanic treatment. Similarly, many atypical antidepressants (e.g., mianserin and rolipram) have not been tested for their antipanic potential. The clinical effects of these treatments and agents (including MAOA- vs MAOB-selective inhibitors) would be of great value in further characterizing animal models for panic disorder based on antipanic treatment response profiles.

Finally, the α_2-adrenoceptor agonist clonidine also has been reported to exhibit antipanic efficacy in humans (Hoehn-Saric et al., 1981; Liebowitz et al., 1981; Uhde et al., 1981,1989). This agent does not possess antidepressant activity. The same thing can be said of clonazepam; indeed, depression is cited as a possible side effect associated with the use of clonazepam in the treatment of other disorders (Pollack et al., 1986; Cohen and Rosenbaum, 1987). Thus, these two agents represent important positive control agents for evaluating potential animal models for their selectivity for the study of antipanic, but not antidepressant treatment effects.

2.2.2.3. SUMMARY—ANTIPANIC, ANTIDEPRESSANT, AND ANTIGAD TREATMENT EFFECTS

The clinical situation reviewed above describes the disorders of depression, GAD, and panic disorder as relatively discrete human syndromes that can be effectively and relatively selectively treated by specific drugs or groups of drugs. Although in pure form and in a small percentage of cases these syndromes may be discrete and distinct, in many cases the overlap between depression, GAD, and panic disorder may be very significant. Indeed, Cameron et al. (1986) have questioned whether it is appropriate that GAD and panic disorder be considered as separate diagnostic entities. It is perhaps not surprising, therefore, that there also exists considerable overlap regarding the efficacy of various treatments in these disorders. Nonetheless, it remains the case that the traditional anxiolytics, such as benzodiazepines and barbiturates, remain the "gold standards" in the pharmacological management of GAD, whereas chronic administration of antidepressant agents (including alprazolam) remains the first line of treatment in the pharmacotherapy of patients suffering from panic disorder.

2.3. "Panicogenic" Treatments

In addition to their spontaneous occurrence in panic disorder patients, panic attacks can be induced experimentally in humans. These experimentally induced panic attacks are very similar to spontaneously occurring panic attacks and can be blocked by the same treatments that reduce the frequency of occurrence of spontaneously occurring panic attacks (Gorman et al., 1985; Beckett et al., 1986; Yeragani et al., 1988). The effects of these treatments in panic disorder patients and normal controls have been used clinically in the study of panic disorder. Five specific treatments have been reported to produce panic attacks in panic disorder patients and, at higher doses, also in normal controls. These treatments are sodium lactate (originally reported by Pitts and McClure, 1967), the β-agonist isoproterenol (Frolich et al., 1969; Rainey et al., 1984), the α_2-antagonist yohimbine (Holmberg et al., 1962; Charney and Heninger, 1982; Charney et al., 1983), the putative 5-HT_{1B} agonist mCPP (Charney et al., 1987b; Murphy et al., 1989), and exposure to 5% carbon dioxide in the inspired air (Gorman et al., 1988; Woods et al., 1988).

3. Criteria for Evaluating Potential Animal Models for Panic Disorder

3.1. Validity of Animal Models for Human Conditions

In his book on depression, Willner (1985) defined three hierarchical levels of validity for which animal models of various human conditions can be evaluated: predictive validity, face validity, and construct validity. A model has predictive validity if there is a high degree of concordance between the effects of various drugs in the model and the effects of these same drugs in the human condition. In this regard, it is a relatively simple task to evaluate the predictive validity of various animal models for a particular disorder if the pharmacotherapy of the clinical condition is well-established and accepted. One simply determines the effects of the available effective and ineffective treatments on the behavior, and evaluates the concordance between the two situations. Beyond the demonstration of predictive validity, a model has face validity if it resembles the etiology, biochemistry, symptomatology, and treatment profile of the clinical condition being modeled. Finally, an animal model has construct validity if it is established that homologous constructs are being studied in the model and in the clinical condition. This requires an empirically supported rationale for believing that the construct in question (in both the model and the clinical situation) is fundamental to the disorder and is not merely an epiphenomenon.

3.2. Operational Definitions of "Antipanic" and "Panicogenic" Treatment Effects Used in the Present Chapter

At the present time, there exists no animal model for human panic disorder based on face and/or construct validity. However, there is a relatively extensive amount of literature (described above) on the pharmacotherapy of panic disorder, depression, and/or GAD. Moreover, there also exist several clinically effective panic-inducing treatments (described above). This allows for the evaluation of animal models based on predictive validity. Critical in this literature is the observation that antipanic effects in humans are dependent upon chronic treatment. Thus, for the remainder of the present chapter, "antipanic" treatment effects will be operationally defined as anxiolytic-like effects that are produced by chronic, but not acute, treatment with clinically effective antipanic agents. Based upon the time course for the clinical management of panic disorder, these "antipanic" effects will be expected to exhibit a 3–4 wk latency to onset, with maximal effects not observed until a minimum of 5–8 wk of treatment. Information provided in Section 4. constitutes a review of the effects of various antipanic and "negative control" treatments in several animal models for anxiety. Section 5 reviews the behavioral effects of panicogenic treatments in these various animal models. Within section 5, "panicogenic" effects will be operationally defined as anxiogenic-like effects that are produced by acute treatment with clinically effective panic-inducing treatments. It should be noted that, in all cases, the animal models were established, at least in part, on the basis of drug responsiveness following acute treatment with traditional antianxiety agents, such as benzodiazepines and barbiturates.

4. Antipanic Treatment Effects in Animal Models of Anxiety

In the animal models of anxiety that have been investigated for their sensitivity to antipanic treatments, the effects of chronic treatment with TCAs (particularly imipramine) have been reported most frequently. In the vast majority of these studies, the maximum duration of chronic treatment is 3 wk. This treatment duration was likely selected on the basis of receptor binding changes and other biochemical effects observed after chronic antidepressant treatment (Vetulani and Sulser, 1975; Banerjee et al., 1977; Peroutka and Snyder, 1980; *see* review by Charney et al., 1981). As discussed below, the majority of these studies resulted in negative findings regarding the potential anxiolytic-like effects of chronic antidepressant treatment. These negative results may not be surprising, however, given that the clinical antipanic effects observed with antidepressant agents often exhibit onset delays of 3–4 wk or greater (as discussed above).

4.1. Conflict Paradigms as Animal Models for Antipanic Treatment Effects

Conflict tests of anxiety, by definition, involve crossed or opposing motivations. That is, such procedures bring two motivational systems, each of which alone would direct behavior in the opposite direction, into conflict. A typical example is provided by the novelty-suppressed feeding (NSF) paradigm used by Bodnoff et al. (1988), where the desire of a food-restricted rat to obtain food in a novel arena is opposed by the simultaneous motivation to avoid extensive exploration of the novel arena. Punishment of operant or consummatory behaviors also has been used extensively to generate behavioral "conflict." The classic example is the Geller-Seifter conflict paradigm (Geller and Seifter, 1960), where the desire of a food-restricted animal to perform an operant task to obtain food is opposed by the simultaneous motivation to avoid an aversive shock. In these paradigms, benzodiazepines, barbiturates, and under certain circumstances, novel anxiolytic agents, such as buspirone, elicit robust and dose-dependent increases in suppressed responding. The anticonflict effects of these clinically effective antianxiety treatments have served as the primary justification for the use of these procedures as animal models for anxiety states and the study of antianxiety agents. Both nonshock conflict paradigms and conflict paradigms involving shock-induced suppression of behavior have been examined as animal models for the study of antipanic treatment effects. These results are summarized below.

4.1.1. Antipanic Treatment Effects in Nonshock Conflict Paradigms

The results of studies examining the potential anxiolytic-like effects of antipanic treatments in nonshock conflict paradigms have been mixed.

4.1.1.1. NEGATIVE FINDINGS—ELEVATED PLUS MAZE TASK

The elevated plus maze task used by many investigators is a modification of the elevated open maze apparatus originally used by Montgomery (1958). This apparatus consists of two open arms and two enclosed arms, with an open roof. The apparatus is configured such that the two open arms are opposite to each other. The maze is elevated to an approximate height of 0.5 m. Rats are placed individually in the center of the maze, and the following measures are scored by trained and blinded observers: (1) number of entries into open vs closed arms, and (2) the time spent in open vs closed arms.

Untreated rats make few open-arm entries and spend little time in the open arm of the maze. Consistent with their antianxiety effects in humans, both benzodiazepine and barbiturate anxiolytics increase the percentage of open-arm entries and the percentage of time spent in the open arm (Pellow et al., 1985b; Pellow and File, 1986). In contrast to the positive effects observed with traditional anxiolytic treatments, chronic treatment (21 d) with the antipanic agent imipramine did not result in an increase in the percentage of time spent in the open arm or the number of open arm entries (File and Johnston, 1987).

4.1.1.2. Negative Findings—Social Interaction Task

The amount of time that pairs of male rats spend in active social interaction (e.g., sniffing, following, grooming, kicking, mounting, jumping on, wrestling and boxing with, and crawling under or over the partner) varies systematically with the test conditions. It is maximal when the rats are in an arena with which they are familiar and that is dimly lit. If the illumination increases or if the arena is unfamiliar, social interaction decreases (File and Hyde, 1978; File, 1988). Consistent with the utility of the social interaction task as an animal model for the study of traditional anxiolytic agents, the "anxiogenic" effects of high illumination and/or low familiarity are reversed by acute treatment with various traditional anxiolytic agents (File, 1980,1987, 1988). In contrast to the positive effects observed with traditional anxiolytic treatments, however, chronic treatment with the antipanic agent imipramine (5 or 15 mg/kg for 21 d) did not result in an increase in social interaction in this paradigm. Indeed, there was actually a tendency for chronic imipramine treatment to reduce social interaction (Pellow and File, 1987). Similar negative findings have been reported for clomipramine (File, 1985) and the nonselective MAOI phenelzine (Johnston and File, 1988). Finally, repeated electroconvulsive shock (ECS) treatment did not affect behavior in the social interaction paradigm (File and Green, 1984).

4.1.1.3. Positive Findings—Novelty-Suppressed Feeding

In the NSF task, food-deprived rats (48 h without food) are placed in a novel environment (relative to the home cage). Food pellets are evenly distributed on the floor. The latency to initiate eating of the food pellets is monitored by trained and blinded observers. When tested in their home cages, animals exhibited very short latencies to the onset of eating (less than 1 min). In contrast, when placed in a novel environment, the latency to onset increased dramatically to 4–5 min. Acute treatment with the benzodiazepines diazepam (2 mg/kg) or adinazolam (20 mg/kg) effectively and completely reversed the effects of novelty on the latency to begin eating (Bodnoff et al., 1988). Similar effects of traditional anxiolytics have been reported by Britton and Thatcher-Britton (1981) and Shephard and Broadhurst (1982).

Although acute treatment with the antidepressants desipramine (10 mg/kg) or amitriptyline (10 mg/kg) failed to affect NSF behavior, chronic (14 or 21 d) treatment with these agents resulted in a significant reduction of the suppression of feeding by the novel environment (Bodnoff et al., 1988). It should be noted, however, that the magnitude of the anxiolytic-like effect at 14 and 21 d of antidepressant treatment (latencies to the onset of eating reduced to 2–3 min) was not equal to the magnitude of the anxiolytic-like effect produced by acute treatment with the benzodiazepines (latencies reduced to 1 min). Unfortunately, the effects of longer periods of antidepressant treatment were not examined by these investigators.

4.1.2. Antipanic Treatment Effects in Shock-Based Conflict Paradigms

4.1.2.1. GELLER-SEIFTER, VOGEL PARADIGMS

The Geller-Seifter paradigm (Geller and Seifter, 1960; Geller et al., 1962) probably represents the "gold standard" for evaluating and investigating the effects of potential anxiolytic agents. The Vogel acute conflict task (Vogel et al., 1971) and its many modifications also have been used extensively in the evaluation and investigation of potential anxiolytic agents. It is therefore quite surprising that the effects of chronic treatment with antipanic treatments have not been examined in these paradigms. This is an area that clearly warrants further investigation.

4.1.2.2. CONDITIONED SUPPRESSION OF DRINKING (CSD) PARADIGM

In contrast to the lack of information regarding the effects of antipanic treatments in the Geller-Seifter and Vogel shock-based conflict paradigms, the effects of antipanic treatments have been studied extensively in the Conditioned Suppression of Drinking (CSD) conflict paradigm (Fontana and Commissaris, 1988; Fontana et al., 1988,1989a,1990; Ellis et al., 1990; Commissaris et al., 1990a). This paradigm is essentially a hybrid of the Geller-Seifter and Vogel paradigms. Test sessions (10 min in duration) in the CSD paradigm consist of repeated alternations of periods in which water intake from a metal tube is not punished and periods in which water intake is punished. Periods of punishment are signaled by the presence of a tone. The repeated test nature of behavioral sessions characterized by signaled punishment availability is analogous to the traditional Geller-Seifter paradigm; the use of water as the reinforcer and the punishment directly of or contingent upon the consummatory response are analogous to the Vogel acute conflict task.

In the CSD conflict paradigm, typical control (i.e., nondrug) sessions are characterized by a relatively stable number of shocks accepted (approx 40 punished licks/session at the 0.25-mA shock intensity) and a relatively constant volume (10–12 mL/d; equates to approx 2500 unpunished licks/session) of water consumed (day-to-day coefficients of variation for punished responding range from 30–50% for individual rats). As expected, acute administration of traditional anxiolytics (barbiturates and benzodiazepines) results in robust and dose-dependent increases in punished responding (Ford et al., 1979; Kilts et al., 1981; Commissaris and Rech, 1982; McCloskey et al., 1987; Commissaris et al., 1988). Acute treatment with the novel anxiolytic agent buspirone usually exerts only minimal anxiolytic-like effects (McCloskey et al., 1987; Schefke et al., 1989). Consistent with the clinical requirement for chronic treatment for maximal anxiolytic efficacy (Goa and Ward, 1986), however, the anticonflict effects of acute buspirone are much greater following chronic buspirone treatment (Schefke et al., 1989).

The CSD paradigm has been evaluated extensively as an animal model for the study of antipanic treatment effects. The effects on CSD behavior of chronic treatment with numerous clinically effective antipanic treatments as well as several important negative control treatments have been determined. In all of these studies, the chronic drug treatment is introduced after behavioral baselines have been established in the CSD paradigm. The subjects are tested in the CSD paradigm 4 or 5 d/wk for a minimum of 5 wk. The chronic drug treatments are administered 2×/d (BID), 7 d/wk; the first drug treatment on any given day is administered immediately after behavioral testing (usually in the AM); the second drug treatment is administered 12 h after the first. Thus, behavioral testing is conducted approx 12 h after the most recent drug injection in the chronic treatment regimen. This chronic posttest treatment regimen was adopted in order to minimize the acute effects of the chronically administered drug at the time of behavioral testing.

Figure 1 illustrates the effects of chronic treatment with the TCA desipramine and the nonselective MAOI phenelzine on CSD conflict behavior. As can be seen, chronic treatment with each agent resulted in a time-dependent increase in punished responding, with a latency to onset of approx 4 wk; maximal anticonflict effects were not observed until approx 6–8 wk of chronic treatment. Acute treatment (10- to 60-min pretreatment) with these agents across a wide range of doses either had no effect on CSD behavior or resulted in a decrease in both punished and unpunished responding (Fontana and Commissaris, 1988; Fontana et al., 1989a).

Just as the onset for the behavioral response exhibited a 3–4 wk latency, discontinuation of chronic desipramine treatment following 5 wk of chronic treatment resulted in a gradual decline in punished responding, reaching control levels only after 3 wk without chronic drug administration (Fontana et al., 1989a). These investigators also reported that chronic phenelzine treatment for 4 wk increased punished responding even when behavioral testing in the conflict paradigm was suspended during the period of drug treatment. Thus, the anticonflict effects of chronic antipanic treatment are not dependent on CSD testing during the course of chronic drug treatment.

Table 1 summarizes the results of studies in which chronic antidepressant treatments exerted anxiolytic-like effects. In both the CSD and in the NSF tasks, chronic treatment with TCAs resulted in a time-dependent increase in punished responding. Chronic treatment with nonselective MAOIs (4 mg/kg

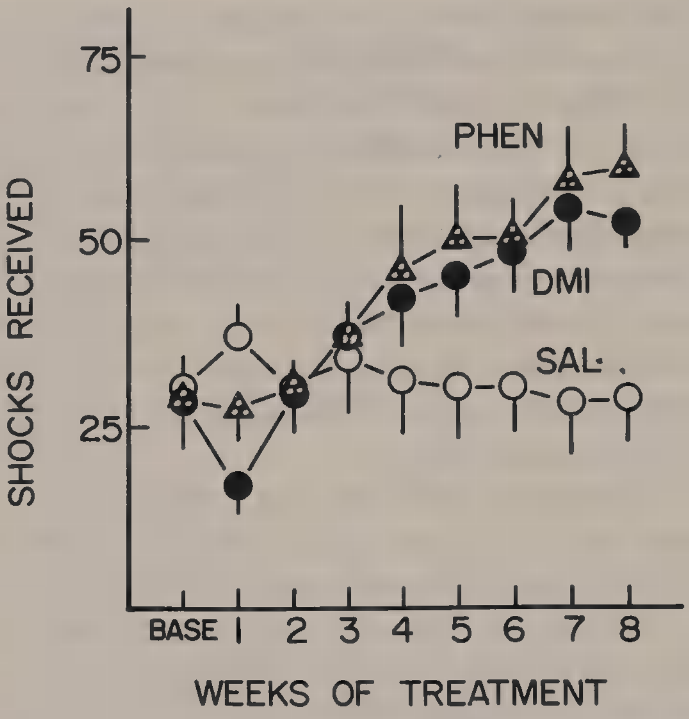

Fig. 1. The anxiolytic-like effects of chronic desipramine or phenelzine treatments on CSD conflict behavior. Plotted are the number of punished responses accepted by rats before (BASE) and during chronic treatment with saline (SAL; open symbols), desipramine (DMI: filled circles, 5. 0 mg/kg, BID), or phenelzine (PHEN: dotted triangles, 4. 0 mg/kg, BID) for 8 wk. Each value represents the mean ± SEM from eight subjects. DMI- and PHEN-induced increases in punished responding were significantly different from SAL-treated rats weeks 4–8 of chronic treatment, *post hoc* least significant differences test following factorial analysis of variance (ANOVA).

phenelzine, BID; 15 mg/kg pargyline, BID) also resulted in a time-dependent increase in punished responding in the CSD paradigm (Fontana et al., 1988, 1989a). Interestingly, chronic treatment with either the MAOA-selective (1.0 mg/kg clorgyline, BID) or MAOB-selective (1.0 mg/kg pargyline, BID) inhibitors failed to increase punished responding, whereas rats receiving both treatments exhibited a time-dependent increase in punished responding (Fontana et al., 1988) much like that seen following chronic treatment with nonselective MAOIs (e.g., 4.0 mg/kg phenelzine, 15 mg/kg pargyline). This finding is somewhat surprising, since it has been proposed that MAOA inhibition alone is sufficient for clinical antidepressant activity (Lipper et al., 1979; Mendis et al., 1981; Aarons et al., 1985). Indeed, MAOA-selective

Table 1
Chronic Drug Treatments
That Produce Anxiolytic-Like Effects on Conflict Behaviors

	Dose administered	Reference(s)
Tricyclic antidepressants		
Imipramine	2. 5 mg/kg, BID	Fontana and Commissaris 1988
Desipramine	10 mg/kg/d	Bodnoff et al., 1988
Desipramine	5 mg/kg, BID	Fontana et al., 1989a Commissaris et al., 1990a
Amitriptyline	10 mg/kg/d	Bodnoff et al., 1988
Amitriptyline	5 mg/kg, BID	Fontana et al., 1989a
Nonselective monoamine oxidase inhibitors (MAOIs)		
Phenelzine	4 mg/kg, BID	Fontana et al., 1989a
Pargyline	15 mg/kg, BID	Fontana et al., 1988
Atypical antidepressants		
Alprazolam	5 mg/kg, BID	Ellis et al., 1990
Trazodone, (minimally effective)	40 mg/kg, BID	Commissaris et al., 1990a
Miscellaneous treatments		
Clonidine	40 µg/kg, BID	Fontana et al., 1990 Commissaris et al., 1990a
MAOI, A and B		Fontana et al., 1988
Pargyline	1 mg/kg, BID	
Clorgyline	1 mg/kg, BID	

inhibition is sufficient for the production of antidepressant-like effects in the learned helplessness (Martin et al., 1987) and differential reinforcement of low response rates (DRL) 72-s (Marek and Seiden, 1988) animal models of depression. Unfortunately, the clinical antipanic efficacy of selective MAOA vs MAOB inhibitors is not presently known.

Consistent with its clinical efficacy in the management of panic disorder, chronic posttest treatment with alprazolam also exerted a time-dependent anticonflict effect. It should be noted that the anticonflict effect produced by this chronic posttest alprazolam treatment is not antagonized by the benzodiazepine antagonist Ro15-1788, whereas the anticonflict effect of acute pretest treatment with alprazolam is antagonized by Ro15-1788 pretreatment (Ellis et al., 1990).

Trazodone is an efficacious antipanic treatment in humans; however, the efficacy of this agent is considerably less than that of imipramine or alprazolam (Charney et al., 1986; Mavissakalian et al., 1987). Consistent with this observation, chronic trazodone treatment produces only a weak anxiolytic-like effect following chronic treatment. Finally, chronic clonidine treatment results in a robust and dramatic anxiolytic-like effect (Fontana et al., 1990; Commissaris et al., 1990a), which is consistent with its clinical efficacy in the treatment of panic disorder (Hoehn-Saric et al., 1981; Liebowitz et al., 1981; Uhde et al., 1981,1989).

Not all antidepressant agents increased punished responding in the CSD paradigm when administered chronically. For example, buproprion, an agent that has been reported to lack antipanic efficacy (Sheehan et al., 1983), failed to increase punished responding in the CSD paradigm (up to 10 mg/kg, BID; Commissaris et al., 1990a). Also ineffective in the CSD paradigm was chronic treatment with the atypical antidepressant mianserin (up to 10 mg/kg, BID; Commissaris et al., 1990a). Again, it is unfortunate that the clinical status of mianserin as an antipanic treatment is not known. However, as with MAOA inhibition data, mianserin treatment in the doses used (10 mg/kg/d) is sufficient to reverse the behavioral deficits associated with the learned helplessness paradigm (Sherman et al., 1982).

Chronic treatment with the traditional benzodiazepine chlordiazepoxide (10 mg/kg, BID for 12 wk or 40 mg/kg/d for 6 wk) failed to increase punished responding (Ellis et al., 1990). A similar lack of anxiolytic-like effect was observed in the CSD paradigm following chronic phenobarbital treatment (80 mg/kg/d; Ellis et al., 1990). It should be noted, of course, that acute pretest challenges with chlordiazepoxide or phenobarbital result in dramatic anxiolytic-like effects (Kilts et al., 1981; McCloskey et al., 1987; Commissaris et al., 1989,1990b); this observation is consistent with their utility in the treatment of GAD.

Finally, β-antagonists, such as propranolol, have been found to be effective in the treatment of phobic anxiety (for example, the anxiety associated with public speaking; *see* Granville-Grossman and Turner, 1966; Turner et al., 1965; Turner, 1983), but these agents have negligible effects in the treatment of panic disorder (Noyes et al., 1984; Gorman et al., 1983). Consistent with its lack of antipanic efficacy, chronic treatment with propranolol (2 mg/kg, BID) also failed to produce an anxiolytic-like effect in the CSD conflict paradigm (Fontana et al., 1989b).

4.2. Nonconflict Paradigms as Animal Models for Antipanic Treatment Effects

Many animal models for studying anxiolytic treatment effects are not based on the concept of experimentally induced "conflict." Included in this category are at least two classically conditioned fear paradigms, the Estes-Skinner Conditioned Emotional Response (CER; Estes and Skinner, 1941) and the potentiated startle response (*see* Davis, 1988). The pentylenetetrazole (PTZ) drug discrimination technique (discriminative stimulus properties of an "anxiogenic" substance; Lal and Shearman, 1980; Lal and Emmett-Oglesby, 1983) is a technique that also should be included in this category of nonconflict animal models for anxiety. Finally, unconditioned behaviors, such as open field defecation (Broadhurst, 1960; Commissaris et al., 1986) and defensive probe burying (*see* review by Treit, 1985), also constitute nonconflict animal models of anxiety. Of these nonconflict paradigms, only the potentiated startle response and the defensive probe burying paradigms have been examined as potential animal models for antipanic treatment effects. The results from these studies, discussed below in detail, have not been promising.

4.2.1. Potentiated Startle Paradigm

It has been known for many years that the acoustic startle reflex can be augmented by presenting the eliciting stimulus in the presence of a cue that has previously been paired with a shock (Brown et al., 1951). This phenomenon, termed the potentiated startle effect, has been replicated a number of times in several laboratories (Anderson et al., 1969; Bridger and Mandel, 1967; Chi, 1965; Davis, 1988; Galvani, 1970; Kurtz and Siegel, 1966; Wagner et al., 1967). The neuronal circuit mediating the potentiated startle response has recently been delineated (Berg and Davis, 1984; Tischler and Davis, 1983). Thus, the potentiated startle paradigm is a procedure that can be used to study conditioned fear from the perspective of its neuroanatomical circuitry (*see* Davis, 1988).

Consistent with its utility as an animal model for anxiety, the potentiated startle response is selectively attenuated or blocked by barbiturate, benzodiazepine, and buspirone-like anxiolytics. Chi (1965) found that the barbiturate amobarbital produced a dose-related reduction in potentiated startle. Similarly, Davis (1979) has shown that the benzodiazepines diazepam and flurazepam produced a dose-dependent reduction of potentiated startle. Moreover, the novel anxiolytics buspirone and gepirone also effectively reduced the magnitude of the potentiated startle response (Kehne et al., 1988). These pharmacological findings, combined with the ease of training and testing, make the potentiated startle response an excellent procedure for studying the effects of traditional anxiolytics on conditioned fear behavior.

To date, there has been only one study in which the effects of an antipanic treatment on the potentiated startle response have been examined. Cassella and Davis (1985) reported that neither acute treatment with imipramine (5 or 10 mg/kg) nor chronic imipramine administration (10 mg/kg for 21 d) exerted anxiolytic-like effects in the potentiated startle response. It should be noted, however, that the 3-wk duration of chronic imipramine treatment may not have been sufficient to produce anxiolytic-like effects. Indeed, the anxiolytic-like effects of chronic imipramine treatment (2.5 mg/kg, BID) in the CSD conflict paradigm were not statistically significant until 4 wk of chronic treatment (Fontana and Commissaris, 1988).

4.2.2. Defensive Probe Burying Behavior

The results of recent studies by Treit and coworkers (Treit et al., 1981,1982; Treit, 1985) suggest that the defensive probe burying paradigm may also serve as a behavioral "model" for the study of anxiety and/or fear behavior. Defensive probe burying behavior consists of four consecutive daily habituation sessions, followed by the test session on the fifth day. During the habituation sessions, the animals are placed into the chamber in groups of four for 30 min. On the fifth day (test session), a wire-wrapped prod is inserted through the wall to protrude 6 cm into the chamber. Animals are placed into the chamber singly for defensive burying test sessions. Upon contact with the prod (usually with the mouth or paw), the animal receives an electric shock (1–6 mA in most studies) for the duration of the contact with the prod (<1 s). The animals are observed for 15 min after shock administration by trained and blinded raters. Three parameters are monitored in this period:

1. The presence or absence of burying behavior (directing bedding material toward or over the prod)
2. The latency to initiation of burying behavior and
3. The duration of burying behavior.

As expected, acute treatment with barbiturates or benzodiazepines reduces the frequency of occurrence of burying behavior (Treit et al., 1981,1982; Treit, 1985; Beardslee et al., 1989, 1990); these agents also reduce the duration of burying in those subjects that do engage in probe burying (Treit et al., 1981,1982; Treit, 1985; Craft et al., 1988; Tsuda et al., 1988; Beardslee et al., 1989,1990). Finally, the latency to onset of this burying behavior is increased in a dose-related manner by benzodiazepine treatment (Beardslee et al., 1990). The novel anxiolytic buspirone has also been reported to suppress defensive probe burying (Treit and Fundytus, 1988), although there is not complete agreement regarding the effects of buspirone in this paradigm (Craft et al., 1986,1988).

In contrast to the robust and reliable effects of antiGAD treatments on defensive burying behavior, recent findings suggest that the defensive burying paradigm is not an effective animal model for the study of antipanic treatment effects. That is, chronic (>5 wk) treatment with the TCAs desipramine or imipramine or the nonselective MAOI pargyline failed to exert anxiolytic-like effects on these parameters (Beardslee et al., 1990). All of these chronic treatments are known to exert prominent anticonflict effects in the CSD paradigm (as described above).

4.3. Summary—Antipanic Treatment Effects in Animal Models of Anxiety

4.3.1. Effects in Nonshock Conflict Paradigms

The nonshock conflict paradigms may be relatively insensitive to the anxiolytic-like effects of antipanic treatments, with no evidence of anxiolytic-like effects seen in the elevated plus maze or the social interaction tasks, and only modest effects observed in the NSF tasks. This is somewhat surprising, since these procedures are highly responsive to the anxiolytic-like effects of traditional anxiolytics, such as the benzodiazepines and barbiturates. However, the effects of long-term chronic treatments (>3 wk) were not investigated. It is possible that the modest efficacy of the desipramine and amitriptyline treatments in the NSF task may increase with a longer duration of chronic treatment. Similarly, it is possible that the 3-wk treatment duration is not sufficient for detecting the anxiolytic-like effects of chronic antidepressant treatment in the elevated plus maze or the social interaction tasks. Finally, the effects of chronic treatment with several important negative (e.g., buproprion) and positive (e.g., clonidine, fluoxetine) control compounds have not yet been examined in these paradigms.

4.3.2. Effects in Shock-Based Conflict Paradigms

As indicated above, the Geller-Seifter paradigm and the Vogel acute conflict task are the standards against which other animal models for the study of antianxiety treatment effects often are judged. It is therefore quite surprising that the effects of chronic antipanic treatment on behavior in these paradigms have not been examined. Clearly, the effects of chronic treatment with antipanic agents on behavior in these paradigms must be determined.

The effects of many antidepressant, antipanic, and negative control compounds on behavior in the CSD conflict paradigm have been investigated. The results strongly suggest that the CSD conflict paradigm (and perhaps other conflict paradigms as well) may be an effective animal model for identifying and studying antipanic treatment effects. The latency to onset for the anxiolytic-like effects of the antipanic treatments typically is 3–4 wk. It is important to note that significant anxiolytic-like effects often are not observed after 3 wk of chronic treatment and that the maximal effect often is not seen until 5–7 wk of chronic treatment. Lacking in the CSD paradigm are reports on the effects of the atypical antidepressant and antipanic agent fluoxetine; also lacking are reports on the effects of chronic posttest treatment with clonazepam, an antipanic benzodiazepine.

In addition to the duration of chronic antipanic drug treatment, there are two additional procedural differences between the studies conducted using the CSD paradigm and many other procedures. First, the studies conducted using the CSD paradigm (and defensive burying) have used female rats. This sex was selected because there was a much greater prevalence of anxiety disorders (including panic disorder) in females than in males (*see* Myers et al., 1984). Although specific studies comparing the effects of chronic antipanic drug treatment in male and female rats have not been conducted, both male and female rats have been studied extensively in the CSD conflict paradigm. No qualitative male vs female differences in drug responsiveness have been observed (Commissaris et al., 1986, 1990b,c). Second, the studies using the CSD conflict paradigm have utilized posttest chronic treatment regimens, whereas most other chronic treatment studies examined the effects of repeated pretest treatment with the agent under investigation. It is possible that pretest chronic treatment regimens fail to detect significant anxiolytic-like effects because the acute effects of the drug interfere with the performance in the behavioral paradigm.

Finally, it should be noted that, in the CSD, Vogel, and Geller-Seifter conflict paradigms, the behavioral suppression is produced as a result of an electric shock. Changes in punished responding resulting from chronic antipanic treatment could also be reflective of changes in sensitivity to shock. To test for this possibility, shock sensitivity determinations should be conducted in subjects receiving the various antipanic drug treatments.

4.3.3. Effects in Nonconflict Paradigms

As mentioned above with respect to the nonshock conflict paradigms, the negative findings with chronic imipramine treatment in the potentiated startle response may be the result of the duration of chronic treatment. However, inadequate duration of treatment does not apply with respect to the negative findings in the defensive burying paradigm. Indeed, the minimum duration of antipanic treatment was 5 wk; moreover, subjects that received chronic desipramine and chronic pargyline treatment had previously displayed significant anxiolytic-like effects in the CSD conflict paradigm (Beardslee et al., 1990). Thus, it appears that the defensive burying paradigm, although an effective animal model for identifying and studying traditional anxiolytics, is not an animal model for the study of antipanic treatment effects. Again, the potential anxiolytic-like effects of chronic antipanic treatment on behavior in several animal models that have been used widely in the study of anxiety (CER, PTZ, drug discrimination, and open field defecation) have not been investigated.

5. Panicogenic Treatment Effects in Animal Models of Anxiety

As described above, panic attacks can be induced experimentally in humans by treatment with several diverse agents. Although not thoroughly evaluated, the potential anxiogenic-like effects of these treatments have been determined in several animal models for anxiety. The results of these studies are summarized below.

5.1. Yohimbine Effects in Animal Models of Anxiety

Of all the clinically effective panic-inducing treatments, the behavioral effects of yohimbine have been most extensively evaluated in animal models of anxiety. The findings from nonshock conflict paradigms are consistent with the anxiogenic effects of this agent in humans. Specifically, yohimbine treatment results in a decrease in open-arm entries, the time spent in the open arm of the elevated plus maze (File and Johnston, 1987; Johnston and File, 1988), and social interaction (Pellow et al., 1985a). The effects of acute yohimbine treatment on the NSF task have not been reported. In nonconflict paradigms, yohimbine also is reported to produce anxiogenic-like effects. This agent increases the potentiated startle effect (Davis et al., 1979) and increases defensive burying behavior (Tsuda et al., 1988). Furthermore, yohimbine exhibits at least a partial generalization to the PTZ "anxiogenic" stimulus cue in the drug discrimination paradigm (Lal et al., 1983). Finally, yohimbine increases urination and defecation in the open field paradigm (Ziebrowska-Lupina and Kleinrok, 1973).

In contrast to the above findings, yohimbine has not been reported to exert anxiogenic-like effects in shock-based conflict paradigms. Indeed, this agent has been reported to exert weak, but significant anxiolytic-like effects in a modification of the Geller-Seifter paradigm (Howard and Pollard, 1988; Sethy and Winter, 1972), the Vogel task (Gower and Tricklebank, 1988), and CSD paradigm (Fontana et al., 1990).

5.2. Isoproterenol, mCPP, Sodium Lactate, and Carbon Dioxide Effects in Animal Models of Anxiety

Isoproterenol treatment did not produce anxiogenic-like effects in either the elevated plus maze (Johnston and File, 1988) or the CSD paradigm (Commissaris et al., unpublished), although intracerebroventricular infusion of the β_2-selective agonist salbutamol did result in a selective anxiogenic-like effect in the CSD paradigm (Commissaris et al., unpublished). Although Benjamin et al. (1990) reported that treatment with the 5-HT_{1B} agonist mCPP and its structural analog trifluoromethylphenylpiperazine (TFMPP) decreased entries into the open arm of the elevated plus maze task, acute treatment with mCPP did not exert a selective anxiogenic effect in the CSD conflict paradigm (Commissaris et al., unpublished). Finally, Johnston and File

(1988) also reported that neither sodium lactate infusions nor exposure to 5 or 20% carbon dioxide in the inspired air resulted in anxiogenic-like effects in the elevated plus maze task. The effects of these treatments have not been examined on other animal models of anxiety.

6. Summary and Conclusions

In the present chapter, potential animal models for panic disorder have been reviewed from the perspective of their sensitivity to (1) antipanic treatments and (2) panicogenic treatments. Clinical antipanic therapy with TCA and MAOI antidepressants frequently requires long periods of chronic treatment, with onset latencies often in excess of 3–4 wk. Thus, many studies purporting to evaluate the potential antipanic responsiveness of animal models may have yielded negative data because of an insufficient period of chronic drug treatment (i.e., 3 wk maximum). Indeed, although the CSD conflict paradigm appears to be able to detect antipanic treatment effects, the latency to onset often is greater than 3 wk. Thus, many of the "negative" findings may need to be reevaluated following a longer period of chronic antipanic treatment. The defensive burying paradigm appears to be insensitive to antipanic treatment effects, however, with TCA and MAOI treatment for up to 12 wk failing to result in anxiolytic-like effects.

Clinically effective panicogenic treatments exert anxiogenic-like effects in some, but not all, animal models of anxiety. Unfortunately, many of these panicogenic treatments have been studied in only one or two of the available animal models. A complete and comprehensive evaluation of the effects of these panicogenic treatments on behavior in the various animal models still needs to be conducted. A critical aspect of such studies with panicogenic treatments would be the demonstration that antipanic drug regimens can prevent or obtund the effects of the panicogenic stimuli. To date, there is only one experiment of this nature. File and Johnston (1987) found that chronic treatment with imipramine (5 or 15 mg/kg; 3-wk duration) did not alter the anxiogenic-like effects of yohimbine in the elevated plus maze.

Finally, the clinical situation regarding both the nosology and treatment of panic disorder vs GAD remains unclear. Resolution of the distinctions and similarities between panic disorder and GAD and the agents that are used to treat these anxiety neuroses will be necessary in order to pursue a more thorough pharmacological evaluation of animal models for panic disorder.

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Methods for Drug Studies in Aggressive Behavior

Michael H. Sheard

1. Introduction

Behavioral pharmacology is the detection, description, and explanation of drug effects on behavior. It can be divided into four subsections:

1. The neurochemical or physiological basis of behavior is investigated by using drugs with a known mechanism of action;
2. Behavior is used to measure the potency, spectrum of action, and structure–activity relationships of drugs;
3. Drugs can be used to produce models of pathological conditions; and
4. The mechanism of action of drugs can be investigated, such as their electrophysiological or neurochemical basis.

These four areas clearly require different methods, and aggressive behavior can be used in all of them.

2. Variables

The variables to be considered in these studies include:

1. Behavioral variables;
2. Physiological variables;
3. Pharmacological variables; and
4. Methodological variables.

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Since animal models for studying aggressive behaviors are detailed in another chapter (Koolhaas and Bohus), this chapter will concentrate on pharmacologic methodology.

2.1. Behavioral Variables

Aggressive behavior consists of complex sequences of movements, postures, and gestures that develop in species-specific forms, and serve functions of self- and species-preservation. The forms of aggressive behavior can be described as combinations of offensive and defensive maneuvers. Seven different types have been described on the basis of their situation and eliciting stimuli: predatory, intermale, fear induced, irritable, territorial, material, and instrumental (Moyer, 1968). It has been postulated that different anatomical and physiological systems underlie offensive vs defensive patterns of these behaviorally differentiated types. At least different anatomical and physiological substrates underlie offensive and defensive forms of agonistic behavior (Flynn et al., 1970; Adams, 1979; Albert and Walsh, 1982).

The natural pattern and frequency of the particular aggressive behavior are variables of great importance, and require careful study and documentation before the administration of any drugs. For example, the administration of drugs at times of a low baseline frequency yields a different result from times of high activity. Aggressive behavior occurs naturally in social groups more rarely than during paired encounters. The variables in social groups include the sex and age composition, presence or absence of pregnant female and newborn, social status relationships, duration of a particular animal's presence in the group, and the duration of the group's existence. The results of drug studies in groups of animals will clearly be different from drug studies of single, isolated, or even paired animals. However, if drug studies are performed in different models, the information from each can be complementary and additive, not simply independent.

Elements of behavior in rodents are usually based on those described by Grant (1963), Grant and Mackintosh (1963), Lehman and Adams (1977), and Dixon (1978 a,b). In cats, relevant behavior has been described by Hess (1928), Hess and Brugger (1943), Hunsperger and Bucher (1967), and Flynn et al. (1970). Ethological studies were applied in behavioral pharmacology by Silverman (1966 a,b) and Grant and Mackintosh (1963). Variables in social groups include the social forces that interact with individual affective and cognitive changes, and these can be perceived in ecologically valid models (e.g., Blanchard and Blanchard, 1977, 1984) that permit observations of both the offensive and defensive forms of agonistic behavior. Ethograms of agonistic and other behaviors in mice have been well described (Krsiak, 1975; Mizek and O'Donnell, 1978; Poshivalov, 1981; Scott, 1966).

2.2. Physiological Variables

Aggressive behavior depends on the integrity of many central nervous system functions, so it is frequently necessary to investigate the effects of drugs on these functions in order to analyze possible mechanisms of action wherever some effect on aggressive behavior is observed. Gross observation may reveal changes in motor or sensory activity, and weight and appetite are important in chronic studies.

A common method for testing motor coordination in rodents is the rotarod. Animals are placed on a slowly rotating rod, and the time taken to drop off is measured. Jiggle cages are used to measure movements. They contain sensitive electromechanical devices to register the movements, and can be used in association with tones and air puffs or electric shock to measure the startle response. This is a relatively simple sensory–motor reflex that can measure reaction time and can be used as a model for anxiety. General motor activity can be measured in an enclosed space crossed by infrared beams. Breaks in the beams are registered electronically and, thus, provide a measure of activity.

Alterations of the setting in which aggressive behavior occurs can often be correlated with physiological changes, which include neurotransmitter and hormonal fluctuations. It is important to know the time parameters of such changes in relation to the pharmacokinetics of a particular drug's action in order to be able to determine the optimal timing of drug administration. Animals in different physiological states metabolize drugs differently. For example, dominant and submissive monkeys differ in response to a tryptophan load (McGuire and Raleigh, 1987). The classic work of Cannon provides an excellent description of the different physiology of flight vs fight and the alarm reaction.

The importance of the strain of animal in addition to the importance of its immediate physiological status also requires attention. For example, wild rats in general show much more aggressive behavior than domesticated rats. Flight and defensive attack are seen to a greater degree also. In mice, strain differences are seen particularly in females. For example, lactating female ICR mice, unlike other strains, such as Rockland Swiss, do not attack female intruders. Sequences and frequency of agonistic components differ between males and females. In addition, such variables as housing, lactation, and type of mating partner are all important.

2.3. Pharmacological Variables

A drug may have a direct effect on aggressive behavior or on motor, sensory, emotional, or motivational systems, and these effects can secondarily inhibit or facilitate aggressive behavior. A direct effect on aspects of social behavior may produce an indirect effect on aggressive behavior and vice versa. Drugs may also affect aggressive behavior by acting on the static or active elements of flight behavior. Drug-induced changes in one animal can alter the behavior of the nondrugged animal, and differences are also seen depending on whether the aggressive or the defensive animal is treated. For example, anxiolytics have been reported to cause intruder mice to be attacked more frequently by resident mice, whereas antidepressants and antipsychotics had no such effect.

Dose range and time of administration of drugs have to be matched to time and frequencies of the items of aggressive behavior under observation. Mode of administration of drug is an important consideration. Acute doses are usually given by parenteral injection. Animals require careful handling to minimize stress. Drugs are frequently given subcutaneously in mice and intraperitoneally in rats. A range of at least three doses is required for acute studies.

Since drugs used for the treatment of disorders of human aggression are usually administered chronically and since the effects of drugs given acutely and chronically can differ markedly, it is usually important to test drug given chronically. In this case, they can be administered mixed with food or in drinking water. It is necessary to measure carefully intake and monitor blood levels.

The agonistic encounters themselves can alter the pharmacological action of a drug. The behavioral effects of specific drugs result from actions on molecular mechanisms in different parts of the brain. Therefore, neurochemical and electrophysiological studies in brain are necessary for understanding behavioral effects. Some excellent reviews concerning the action of drugs on agonistic behaviors are available (Miczek, 1987; Miczek and Barry, 1976; Miczek and Krsiak, 1979; Olivier et al., 1984).

2.4. Methodological Variables

2.4.1. Operant Methodology

Operant methodology was in vogue for a while and had certain advantages. Thus, intermittent reinforcement schedules could be understood as inducing frustration and, thereby, stimulating aggressive responses. Animals most commonly used were pigeons, and a pecking response at a mirror was the usual measure of aggressive responding. This could be counted electronically. An advantage of this methodology is that another behavior, such as food reinforced responding, could be measured at the same time in the same animal and provide a control for specific action of a drug on aggression.

Another method utilizing operant conditioning to study the action of drugs on aggression is called the extinction paradigm. Animals are trained singly to press a lever for food reward, and are then paired and subjected to nonreward. In this situation, aggressive behavior is elicited, and a dominant animal and a submissive animal usually emerge from the contest. Drugs have different effects when given to either the dominant or submissive animal, or can be given to both. Many different types of animal can be used in this paradigm, for example, rats (Miczek and Barry, 1974) and pigs (Arnone and Danzer, 1980a). In the latter case, an important methodological principle can be illustrated (namely, that the conditions of testing during extinction can alter the action of drugs). For example, when paired pigs were tested during extinction with access to response panel and food bowl, aggressive behavior, such as pushing and biting episodes, was not altered by diazepam. On the other hand, when access to response panel and food bowl was not permitted, there was an increase in aggressive behavior with diazepam (Arnone and Danzer, 1980b).

2.4.2. Aversive Methodology

Aversive methods of producing aggressive behavior have been used extensively in studying the action of drugs. Shock-induced fighting was first described by O'Kelly and Steckle (1939), and extensively developed by Ulrich and Azrin (1962). This type of irritable or reflexive fighting has been demonstrated in mice (Tedeschi et al., 1954, 1969), hamsters, rats, and cats (Ulrich et al., 1964), and monkeys (Azrin et al., 1963), as well as in some nonmammalian species. The type of behavior that results when pairs of animals, usually rats, are placed in a small enclosure and given painful stimuli consists of rearing, striking with forepaws, and snout biting. It is typical of defensive aggression (Blanchard and Blanchard, 1977). It can be produced by stimulation of the back or tail, as well as by the more usual method of stimulating the feet.

Animals used are typically rodents, for example, experimentally naive rats of Sprague-Dawley strain obtained from Charles River Co. (Kingston, NY), weighing between 280–350 g at the time of testing, and paired on the basis of weight. The rats are maintained in an animal colony room three to a cage of nonfighting members on a 12–12 light–dark 7:00 AM–7:00 PM cycle. Rat chow and water are freely available.

The shock-elicited fighting apparatus consists of a Plexiglass™ box 30 × 28 × 24 cm with a steel grid floor of 0.3-cm parallel bars. The size of the test box is important in determining the frequency of boxing behavior vs escape. The smaller the dimensions, the greater the frequency of boxing. This box is housed in a dimly lighted sound attenuated chamber.

Behavior is observed from a darkened room. Electric shocks are delivered via a scrambler switch to the grid floor. Shock intensity, shock duration, and intershock intervals are controlled by solid-state circuitry.

Behavioral testing consists of subjecting pairs of rats, after 5 min of adaptation to the test cage, to a series of shocks, usually 30 at 0.5-s duration with a 7-s intershock interval. Reducing the intershock interval increases the amount of boxing, whereas increasing the intershock interval tends to decrease it. The goal is to obtain two groups of paired rats that will have similar mean levels of fighting. The level of fighting should be around 50% of the shocks, so that an increase or a decrease in drug effect can be measured. A reasonable shock intensity is 1 mA. There needs to be a minimum of six pairs of rats in each group. One group receives the drug, most commonly given by ip injection, and the other group a placebo. Drugs can be given chronically this way, or by addition to drinking water or food. In the latter cases, however, drug levels should be obtained in some representative animals.

An alternative method is to use a threshold procedure subjecting pairs of rats to ascending and descending shock intensity trials, e.g., 0.05, 0.10, 0.25, 0.50, 1.00, and 1.50 mA, taking intensity at which 50% boxing occurs. An alternating ascending and descending series of shock intensities can also be used with single rats to test for flinch threshold as a method of monitoring pain perception that might result from certain drugs. Flinch threshold refers to the shock intensity at which the rat flinches on 50% of the shocks. A useful technique is to wipe the feet of the rats clean with glycerine prior to placing them in the test cage. In addition, it is good practice to handle the rats frequently and have them get used to injections prior to the actual experimental procedures.

A convenient method of giving rats ip injections consists of holding the rat vertically in a gloved left hand (for right-handed people), with thumb and index finger under the two front forepaws and the palm of the hand supporting the back. The injection is given with a syringe held in the right hand. A small 1-mL tuberculin syringe is a useful size. The usual amount of total injection is kept to 1 mL or less. The plunger is withdrawn slightly, and the syringe is observed to ensure there is tension and nothing is withdrawn. The junction of upper two-thirds and lower third of the abdomen below the rib cage is a convenient site.

Drugs are given in isotonic solution or in nonirritant vehicles. If doubts exist about irritant quality of the drug or vehicle, several rats should be injected with different strengths, and a postmortem inspection of the peritoneal cavity performed. The various aversive experimental methods, such as electric shock, isolation (Ginsberg and Alee, 1942), and physical provocation (Scott, 1966; DaVanzo et al., 1966), have led to results that depend on the particular type of aversive stimulation. It also happens that there are important interactions between the aversive stimulus itself and drugs. These complications have led to the extensive use of what is called the resident-intruder paradigm (Grant and Mackintosh, 1963).

2.4.3. Resident Intruder Methodology

In the resident intruder method, used extensively with rodents, the cage housing the resident becomes the test cage, and the experiment consists of placing an intruder into it. Observations of agonistic behavior are recorded in a variety of ways from simple observations to detailed computer analysis of videotaped sessions. The elements of behavior are those based on descriptions by Grant and Mackintosh (1963), and include such categories as nonsocial activity, social investigation, flight, defensive activity, attack, threat, and displacement. Scores for these items are obtained and compared with scores obtained following administration of drugs. Drugs may be administered to either the resident or the intruder, or to both.

The resident can be a single animal of either sex, and the intruder or the resident may have a mate with offspring. It is customary for offspring to be removed during an experimental session. This is a common paradigm for studying the effects of drugs on female aggression through fertilization, pregnancy, postpartum, and lactation. The variability of the intruders' behavior can be reduced by using nonaggressive naive males, olfactory bulbectomized males, or castrated males. Drugs can have opposite effects on the interactive behavior, depending on whether they are given to the resident or the intruder. Common dose ranges for several drugs are 5, 10, and 20 mg/kg, e.g., for chlordiazepoxide, imipramine, and d1-propranolol, but 0.25, 0.5, and 0.75 mg/kg for haloperidol. Doses ideally should be as close to the human dose range as possible.

2.4.4. Neural Stimulation Methods

2.4.4.1. ELECTRICAL STIMULATION

Electrical stimulation of brain at sites known to elicit aggressive behavior can be used to investigate the action of drugs. Drug effects on responses to stimulation can be measured in three ways. A threshold change can be measured by changes in the intensity of stimulation needed to produce a response, changes in latency between stimulation and response can be measured, and aggressive behavior can be rated along several dimensions, such as resistance to handling, vocalization, reaction to a blunt probe in front of snout, reaction to a blunt probe against side, and biting.

In this method, animals are anesthetized and placed in a stereotaxic apparatus. Stereotaxic parameters for electrodes and cannulas for injecting drugs are derived from appropriate atlases, e.g., for the rat (Pellegrino and Cushman, 1967). Two types of stimulating electrodes are frequently used. Monopolar electrodes are made from stainless-steel wire etched to a sharp point in an electrolytic bath of hydrochloric acid and insulated in coats of baked priming paint. The maximum outside diameters of these electrodes is 500 μm, and the tips are bared of insulation for 0.25 mm. Bipolar electrodes consist of two stainless-steel Teflon™-coated .005-in. wires tightly twisted together covered with a thin layer of epoxy resin and inserted into a #23-gage hypodermic needle with the point filed off. The cut end of the two wires serves as the stimulating tip (tip separation is approx 50–100 μm), and the maximum outside diameter of the electrode shaft is 350–400 μm. An insulated #23-gage needle with the tip filed off can serve either as a monopolar electrode or for the microinjection of chemicals.

Electrodes are implanted into appropriate sites and set into a dental cement cap on the animal's skull locked on by screws. For stimulation, the electrodes are connected to appropriate stimulation circuitry. For example, stimulation can be provided by a Tektronix 162 wave generator and 161 pulse generators connected to cathode follower amplifiers, and fed through stimulus isolation units that isolate the current wave pulse. This biphasic pulse can vary from 0.10 to 0.5 ms half-cycle duration at a frequency of 62.5 Hz. Normal stimulation intensities range from 0.10 to 0.50 mA (peak to peak). A 50,000 Ω resister is placed in series with each stimulator to approximate constant current conditions, and a voltage drop across a 100 Ω precision resistor placed in series with the stimulating leads is used to display the current on an oscilloscope.

Stimulation is delivered in trains of biphasic pulses lasting 20–30 s with 3 min between trains and the intensity varied up and down in 0.1-mA steps to obtain threshold intensity. Once a reliable intensity is found, trains of stimuli at the same intensity are programmed, and latencies to response are measured.

It is possible to investigate the action of drugs on electrically elicited aggressive behavior from primary neural substrates, such as medial and lateral hypothalamus, ventromedial midbrain, or periaqueductal gray, as well as on the modulation of such behavior from other neural substrates, such as amygdala, hippocampus, or such neurotransmitter substrates as the raphé region or locus ceruleus. For concurrent stimulation, the two stimuli are delivered by two independent pulse generators to cathode follower amplifiers and led through two pairs of stimulus isolation units to two relays, which are then connected to the electrodes. When, for example, the raphé region and the periaqueductal gray are stimulated, the biphasic pulses are locked, so that the onset of stimulation to the periaqueductal gray follows the onset of stimulation to the raphé. Delays of 2–12 ms have been used without altering the effects of dual stimulation.

2.4.4.2. MICROINJECTION EXPERIMENTS

Drugs may be administered directly into brain by microinjection techniques. Cannulae can be made from 23-gage needles turned down and threaded to accept an inner cannula made from 30-gage needles that protrude 0.5 mm. The outer cannula is implanted using stereotaxic techniques, and secured to the skull with screws and dental cement. They are placed so that tips are

0.5 mm above intended site. The 30-gage cannula is connected via P 10 polyethylene tubing to a 10-mL syringe mounted in a micromanipulator. One to 2 mL vol is delivered manually over a period of 30 s, or solutions can be infused at a rate of 1 μL/min with a Harvard infusion pump. Vehicle controls are used at all sites to estimate nonspecific damge. 5,7-dihydroxytryptamine is used to deplete brain serotonin, and 6-hydroxydopamine is used to deplete catecholamines. Pretreatment with desmethylimipramine (25 mg/kg) given ip 45 min before injection of 5,7-dihydroxytryptamine protects the noradrenaline system from destruction, and similarly, fluoxetine can protect the 5-hydroxytryptamine system from destruction by 6-hydroxydopamine.

2.4.4.3. Microiontophoretic Experiments

Microiontophoresis has some advantages over other methods for administering chemicals to the brain. It enables precise amounts to be ejected and stopped quickly, because the emission is controlled by constant current. It allows precise localization and minimizes damage because of small tip size. Since only ions are passed, the technique avoids the problem of major changes in volume or toxicity. Drinking behavior has been elicited in rats using carbachol this way, and temperature changes have been elicited using a gel-filled cannula chronically implanted.

For example, to investigate the effects of lithium on neural substrates for aggressive behavior, a cannula serving as a monopolar electrode is chronically implanted. A glass micropipet pulled with a few strands of fiberglass in the tip is filled with molar lithium chloride. This measurement allows the tip to emerge from the cannula at the same level as the site of stimulation. A short length of insulated platinum wire free of insulation for 0.5 mm at the tip is inserted into the micropipet. The other end is soldered to a 20-gage wire attached to a bridge connection as part of the cement skull fixture. A second wire connection is attached to a screw in the skull. Electrical current is delivered via a constant direct-current stimulator. Lithium is ejected by means of a positive current applied to the pipet lead. The following formula can be used to calculate the amount of lithium ejected:

$$M = (n\,C\,T)/(F\,Z)$$

M = mols ejected
n = transport number
C = current in amps
T = time of ejection in s
F = Faraday's constant $(9.65 \times 10\ )$
z = equivalents/mol

If n, which is a measure of ejection efficiency, is assumed to be 1, the calculation will yield the maximum amount of drug that can be ejected by a given current for a given time. It should be noted that there can be increasing resistance to the passage of ions over time with this method.

Sites of electrode and cannula placement are determined by histological techniques. In brief, under deep pentobarbital anesthesia, the rats are perfused transcardially with physiological saline and 10% buffered formalin, after which brains are left for at least 1 wk in formalin solution. Before sectioning on a freezing microtome, the brains are placed in a solution of 40% sucrose and 20% formalin for several days to reduce ice crystal artifact. For routine localization of electrodes and cannulas, alternate sections are stained by Cresyl violet for cells and by the Woelcke technique for myelin sheaths. Localization of micropipet can be performed by making a small lesion with an anodal current (e.g., 20 nA for 20 min). Brain sections need to be examined closely for nonspecific damage and damage to fibers of passage. Nonspecific lesion effects are not usually correlated with the behavioral effects of stimulation, and specific behavioral effects may be correctable by injection of relevant agonist or antagonist drugs. Dose-dependent effects are usually compared with effects of placebo vehicle administration. Since the action of drug includes both facilitatory or inhibitory effects, initial test thresholds should be set to allow for changes in both directions. It is important to make careful observations on other behaviors that may be elicited from the same electrode and on behaviors that may occur between stimulation periods. Finally, it should be noted there are important species differences in the pharmacology of electrically elicited attack.

2.4.5. Hormone Experiments

Aggressive behavior is regulated in part by hormones. The action of hormones on aggressive behavior can be studied in a number of ways. Naturalistic observations can be made, and correlated with hormone levels in urine, blood, or cerebrospinal fluid. Hormones can be administered acutely or chronically, and observations made sequentially. Since gonads respond rapidly to changing blood levels of sex hormones, it is the usual practice when investigating the action of sex hormones, or drugs that influence them, to perform orchidectomy or ovariectomy first. Then appropriate hormone levels can be maintained constant at whatever level is required.

A common method for the chronic administration of hormones is to use silastic tubing cut into small lengths, for example, 1–2 mm, and packed with the desired hormones, for example, testosterone. The tubing is then sealed off at both ends and implanted SC under anesthesia. A common site is on the dorsal surface around the shoulder region.

2.4.6. Social Group Experiments

In social group drug experiments, the species used, size of enclosure, and number of members of different age and sex can vary enormously. This being the case, only certain principles will be mentioned. Methods of drug administration and physiological sampling have to be used that minimize stress and disturb behavior the least. For example, a method has been described for primates whereby they are entrained to enter a capture and isolation unit (Smith, 1981) whenever an entry door is opened. Furthermore, animals that are to receive drug treatment are trained by operant conditioning techniques to present an arm for intramuscular injections (Byrd, 1973, 1977). Selected post-injection time-periods are sampled for behavior, including aggressive items (Altmann, 1974), and stored in digital format for later storage and analysis (Smith and Begeman, 1980).

2.4.7. Statistical Analysis

Nonparametric Kruskal-Wallis tests can be used to assess variance in behavioral measures over different treatment groups. However, where possible, a within-group comparison, a repeated measures design, is preferable to a between-group comparison. Each subject is exposed once to every stimulus animal in rotation until all possible combinations are completed.

Following two- or three-way analysis of variance, paired comparisons can be made with Mann-Whitney U test or Fisher's exact probability test. When there is considerable heterogeneity of variance, it is sometimes helpful to convert output data to natural logs prior to the analysis of variance. The analyses of multiple items and changes in behavior with drugs are very complex and difficult problems. Some initial approaches to them have been reported (Poshivalov and Khodko, 1984; Haccou, 1986).

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Animal Models of Human Aggression

J. M. Koolhaas and B. Bohus

1. Introduction

Much of our present understanding of human biology has been acquired directly from animal experiments. Animal research has strongly enhanced our understanding of neurochemical, neuroendocrine, and genetic factors in normal and pathological functioning of human beings. However, the role of animal research in the study of human behavior and psychiatry is still controversial, despite the frequent use of animal experimentation in the preclinical screening of psychoactive substances. This controversy is mainly owing to an overemphasis of the direct clinical relevance of animal models. It is necessary, therefore, to discuss the rationale of animal models and their limitations first, before embarking on a detailed description of animal models on human aggressive behavior. The evaluation of the usefulness of animal models of aggression needs to be closely linked to the major purpose of developing the model. In a description of the use of animal models in the study of human affective disorders, McKinney (1989) discusses four categories of animal models:

1. Behavioral similarity models: models developed to simulate a specific sign or symptom of the human disorder;
2. Theory-driven models: models developed to evaluate etiological theories of human psychopathology;
3. Mechanistic models: models developed with the primary purpose of studying underlying mechanisms; and
4. Empirical validity models: models developed to permit preclinical evaluation of treatment methods.

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Although these categories may often overlap, researchers wishing to utilize animal models in the study of aggression have to consider their basic scientific questions and goals in the selection of the wide variety of aggression models available in the literature. These basic scientific questions depend, of course, on the theoretical insights into the biological basis of aggressive behavior.

Aggressive behavior can be observed in a wide variety of social contexts. Usually, the performance of aggressive acts is highly adaptive to the individual and/or the species both in humans and animals (Huntingford and Turner, 1987). Much of the scientific interest in aggression, however, is motivated by the violent, hostile, and presumably less adaptive forms of aggression observed in everyday human life, and clinically across a wide spectrum of psychiatric and neurological disorders. Although most of the animal research is focused on aggression as a natural and adaptive behavior, the human studies are mostly aimed at the origins and/or control of violence and criminal behavior. The relationship between these two aspects of the spectrum is still far from clear, which is a major obstacle in the integration of animal research with data on human aggression. Although it is not the main purpose of this chapter to give an extensive review on the biology of aggression, we will briefly summarize the most important factors.

1.1. Kinds of Aggression

It has long been recognized on the basis of animal research that different kinds of aggression seem to exist. Moyer (1968) offered a kind of taxonomy of aggression mainly based on the diversity of eliciting stimuli. Most of the different types of aggression will be presented in this chapter as separate animal models. In their discussion of animal analogs of human aggression, Knutson and Viken (1984) suggest, however, that two of these, namely instrumental and irritable aggression, are particularly relevant for analyses of human aggressive behavior. Instrumental aggression is a form of aggressive behavior instrumentally used to obtain desiderata. Irritable aggression is induced by aversive (social) events. This distinction between two types of human aggressive behavior may be analogous to the distinction between offense and defense made by Blanchard et al., (1977c) and Lehman and Adams (1977) on the basis of animal experiments. Offensive behavior is displayed by a dominant resident when an unfamiliar male conspecific is introduced into the home area. Defensive behavior is performed by an animal that is attacked by either a dominant male or a predator. The offense–defense distinction plays a prominent role in understanding the biology of animal aggression.

A second important development in aggression research can be found in recent studies on the relationship between aggression and coping. Benus (1988) extensively studied the behavioral differences between aggressive and nonaggressive male mice and rats in a wide variety of challenging situations. High levels of aggressive behavior appeared to be related to a good, active shock-avoidance behavior (Benus et al., 1990) and a strong intrinsic behavioral control. This intrinsic control was expressed as routine-like behavior, i.e., aggressive males did not react to changes in an otherwise familiar social and nonsocial environment. On the basis of these experiments, it was concluded that aggressive behavior was part of a more general active coping strategy. The antipode of this active, problem-focused coping style was a passive or emotion-focused coping style. This was expressed as the absence of aggressive behavior in a social situation and passivity in other challenging environmental conditions. In this view, it was not surprising that aggressive behavior was not only related to high levels of testosterone, but also to a high reactivity of the sympathetic nervous system and the adrenal medulla. A low level of aggressive behavior, i.e., the passive strategy, was related to a higher reactivity of the pituitary of adrenocortical axis and the parasympathetic branch of the autonomic nervous system, and low levels of plasma testosterone (Bohus et al., 1987).

This distinction between active and passive coping styles in relation to individual levels of aggression was also made by Henry and Stephens (1977), who summarized different reaction patterns to stress both in animals and in humans. The active coping style was similar to the fight–flight reaction described by Cannon (1929), whereas the passive style was originally described by Engel and Schmale (1972) as conservation withdrawal response. These different coping styles seem to be analogous to the distinction in human type A and type B personalities used in cardiovascular psychophysiology (Friedman and Roseman, 1959). The type A personality is described as aggressive, hostile, and competitive, and is physiologically characterized by a high sympathetic reactivity. In conclusion, it seems that aggression can be considered as an expression of a more general tendency to active environmental control. This view might lead to a different insight into the physiological mechanisms underlying aggressive behavior and its pathology.

1.2. Hormonal Factors

Traditionally, on the basis of animal experiments, high levels of aggressive behavior have been linked to high levels of plasma testosterone (Rose et al., 1971). The relevance of this relationship to human violence and criminal behavior is still heavily discussed (Burrows et al., 1988). However, with the present state of knowledge, it seems to be an example of an unwarranted extrapolation of animal experiments to the human situation (Benton, 1983). This may be because of two confounding factors. First, various kinds of aggressive behavior may be differentially dependent on testosterone. Second, experiential factors strongly interfere with the degree to which aggression depends on plasma testosterone levels (Luttge, 1971). In human beings, such experiential factors might play a more prominent role in overt aggressive behavior. The same uncertainties seem to exist for the role of estrogens and progestins in the suggested higher incidence of violence in women as part of the premenstrual syndrome (Dalton, 1980).

1.3. Genetic Factors

The possible role of genetic factors in human violent behavior has long been recognized. In the early 1960s, several studies focused on the presence of genetic abnormalities. In a population of prisoners, a higher incidence of XYY males was found than in a control group (Goldstein, 1974). Subsequent studies in a noncriminal population of XYY males revealed that these males were probably more impulsive in their decision-making, which in turn might lead to aggressive behavior (Zeuthen et al., 1975). The genetic basis of aggression has also been the subject of many animal experiments (van Oortmerssen and Bakker, 1981). Although no animal models of the XYY syndrome exist, recent evidence suggests that part of the biological variation in aggressive behavior is owing to genetic variation at the Y chromosome (Benus et al., 1991).

1.4. Central Nervous Factors

There is a large body of literature on the central nervous mechanisms of aggressive behavior in animals. The specific involvement of various limbic and midbrain structures seems to depend on the kind of aggressive behavior in question. For example, lesions in the ventromedial hypothalamus increase irritable aggression, but have little or no effect on offense (Oliver, 1977; Adams, 1971). Similarly, in a review on the neurology of human aggression, Weiger and Bear (1988) suggest that distinct neurological lesions or abnormalities may result in distinct types of aggressive behavior. In the human literature, much attention is paid to the so-called dyscontrol syndrome or episodic rage, which is probably attributed to seizure activity in certain brain areas (Weiger and Bear, 1988; Gedye, 1989). Also in chronic neuropsychiatric patients, some aspects of violence seem to be the result of a history of seizures or frontal cerebral lesions (Heinrichs, 1989). The most consistent findings of central nervous functioning in aggressive behavior, however, involve central serotonin (5-HT). Low concentrations of 5-HIAA, a metabolite of serotonin, in the cerebrospinal fluid have been found consistently in highly violent, aggressive, or suicidal patients (Burrows et al., 1988; Sheard, 1988). These findings are supported by many animal studies and form the basis of a pharmacological antiaggressive treatment using 5-HT agonists (Olivier et al., 1987).

It is suggested that several other neurotransmitters, including noradrenaline, dopamine, and GABA, are involved in the control of animal aggressive behavior (Sheard, 1988). Their role in human aggression is far from clear so far (Brown et al., 1979).

2. Animal Models

Aggressive behavior is a highly adaptive behavior that can be observed in almost all animal species. It may serve to protect the home range and its resources against intruders; it may function as defense against predators or attacking conspecifics, and it can be used instrumentally to obtain desiderata. This means that aggressive behavior can be observed in a wide variety of natural and seminatural conditions. The various models mainly differ in the environmental conditions used to induce aggressive behavior. Occasionally, aggression induced by specific manipulations of the brain is used as a model of aggressive behavior.

2.1. Resident–Intruder Paradigm

An animal model in which aggressive behavior can be observed reliably is the so-called resident–intruder paradigm. This model is based on the fact that many animal species will defend their home range against unfamiliar intruding conspecifics. Although most vertebrate species can be used, the principles of the paradigm and the most important variables will be illustrated with our own experiments on male rats. Adult male rats (4–6 mo of age) are housed individually in relatively large observation cages (floor area about half a square meter) for a period of at least 1 wk. This period allows the resident to familiarize with the home cage. When, after this period, an unfamiliar male conspecific of the same strain and weight is introduced into the home cage, the resident will attack the intruder, and a vicious fight will develop. Usually, the resident will be the victor of this social interaction.

This resident–intruder paradigm allows an analysis of aggressive behavior in its full richness. A wide variety of social introductory behaviors, threatening elements, and overt aggressive behaviors can be observed. A description of these elements in male rats is given in Koolhaas (1980). For an extensive description of these behaviors in other species, the reader is directed to the standard ethological literature.

An attractive aspect of this paradigm is that it allows not only the study of offensive behavior in the resident, but also defensive behavior in the intruder male. The paradigm can be considered as a well-controlled laboratory test for both offensive and defensive aggressive behavior. The paradigm also allows an approach of more complex situations. When the resident and the intruder are kept together for a longer period of time, a stable dominance relationship will develop. The complexity of the situation can easily be further increased by using more males and introducing females as well in larger cages. Such colony situations are frequently used to study social relationships in a number of animal species. Using the resident–intruder paradigm, one should control for a number of variables, because they can strongly affect the outcome of the experiments.

2.1.1. Species and Strain

Although all vertebrates may behave aggressively in a resident–intruder paradigm, species and (within a given species) strains may differ in their tendency to perform these behaviors. The differences between species are largely determined by the specific ecological niche to which they are adapted. Strain differences seem to reflect the genetic and ontogenetic varability within a species. However, strain differences are mainly restricted to the probability of showing aggressive behavior. All strains potentially show the full range of species-specific behavior patterns that are known from wild rodent species (Boice, 1972).

2.1.2. Housing

One of the fundamental features of the resident–intruder paradigm is that the resident is kept and tested in its home cage. A certain degree of familiarity or "territoriality" is essential to elicit offensive behavior. This "territoriality" depends on a number of variables. First, cage size is important. Adams (1985) showed that attack behavior was more frequent and dominance relationships were established in the larger cage sizes. A suitable cage size for a male resident rat is about half a square meter. Second, territoriality and familiarity are largely based on olfactory cues (Mugford and Nowell, 1970). Because of the importance of olfactory cues, great care should be taken in cleaning cages in the course of experiments. Novelty from a cleaned bedding strongly interferes with the probability of offense.

2.1.3. Social Experience

A variety of experiential factors affect the quality and intensity of the aggressive interaction. Repeated encounters with intruders gradually shorten the attack latency and change the pattern of the behavior. In particular, the more introductory behavioral elements, like social investigation, are reduced with repeated winning experience (Blanchard et al., 1977b). Defeat strongly reduces the tendency of offensive behavior (van de Poll et al., 1982). Several studies have shown that sexual experience enhanced offensive behavior (Flannelly et al., 1984). This phenomenon is often used to facilitate behavior by housing each resident male together with a female. In order to prevent pregnancy in the female and consequently confounding factors because of changes in social relationship between male and female, the latter is usually sterilized by ligation of the oviducts.

2.1.4. Intruder

The amount and quality of the offensive behavior performed by the resident male strongly depends on the behavior of the opponent. It is necessary, therefore, to standardize the intruder as much as possible. There are several ways to achieve standardization.

In order to be sure that the resident wins the social interaction, the intruder must be of the same or a slightly lower body weight as the resident male. One can also use a different and less aggressive strain. However, we prefer to use the same strain, because, in this way, the level of offense of the resident seems to be more adapted to the level of defense in the intruder.

An important source of variation is the fact that not only the resident male, but also the intruder learns with repeated social interactions. This means that the intruder and, hence, the stimulus situation are not constant in the successive experiments.

In experiments where changes over time are critical, each test should be performed with a naive intruder. In less critical experiments, one can standardize the intruder by giving it defeat experience once or twice before being used in the experiment.

2.1.5. Field of Application

The resident–intruder paradigm is widely used in many species and in many scientific disciplines, primarily as a model to study the central nervous and neuroendocrine mechanisms of aggressive behavior. Manipulations of the amygdala (Luiten et al., 1985), the hypothalamus (Kruk et al., 1979), midbrain structures (Mos et al., 1982), and frontal cortex (de Bruin, 1990) affect behavior in this paradigm. Moreover, neuroendocrine changes in both the resident and the intruder are well documented (Schuurman, 1980), whereas other studies also focus on the causal relationship between these neuroendocrine parameters and subsequent offensive or defensive behaviors (Leshner, 1975). Apart from these mechanistic aspects, the model is frequently used in pharmacological studies to test possible effects of drugs on aggressive behavior and social skills (Olivier et al., 1987).

Finally, the model is used in research aimed at the study of the behavioral and physiological consequences of aggressive behavior, i.e., social stress research. The model allows a detailed study of the way in which social stimuli, such as winning or losing of social interactions, induce affective disorders, such as depression (Koolhaas et al., 1990), or affect the cardiovascular system (Fokkema and Koolhaas, 1985), the immune system (Bohus and Koolhaas, 1990), and so on.

To what extent the model is useful in the study of pathological forms of aggression remains an open question. It may well be a good model for some aspects of human aggressive disorders, in particular in situations in which there is no escape possibility for the intruder.

2.2. Maternal Aggression

Aggressive behavior displayed by females in defense of their young is a well-known phenomenon in a wide variety of species. It has been studied in the laboratory mainly in mice and rats. Basically, the same setup is used as in the resident–intruder paradigm. After mating, females are housed in individual observation cages of about 50 × 50 cm with a bedding that allows them to build a nest. Tests generally begin by placing an unfamiliar male into the female's cage and are continued for 10 min. During the test, pups, if any, are present.

The most important variable in maternal aggression is the time after parturition. The level of aggressive behavior is very low in the early phases of gestation and increases rapidly in the last week of gestation. Aggressive behavior is highest in the first 2 wk of lactation, after which it gradually declines until weaning (Olivier and Mos, 1986b). Defensive behavior in the female shows little change in the course of gestation. A list of behavioral elements that can be observed in a maternal aggression test in rats is given in Olivier and Mos (1986a).

2.2.1. Presence of Pups

Maternal aggression strongly depends on the presence of the pups and the ability to nurse the pups. The attacks on intruders decline significantly within 2 h after removal of the pups. Garland and Svare (1988) showed that suckling stimulation was essential for both the initiation and maintenance of male-directed aggression in the postpartum period.

2.2.2. Familiarity with Cage

Also, in maternal aggression, familiarity with the test environment is important. Svare et al. (1981) showed that aggression was significantly reduced when testing took place in a neutral environment as compared to testing in the home cage. This means that the test cage should preferably not be cleaned in between successive tests.

2.2.3. Intruders

Usually, adult males are used as opponents in the maternal aggression paradigm. However, it appears that the opponent is not a very important variable. Svare et al. (1981) found no significant differences in attack behavior between male and female intruders and young or adult intruders of both sexes.

2.2.4. Species/Strain

Maternal aggression can be observed in a wide variety of animal species. Scientific attention, however, is mainly focused on mice and rats. Although all strains show maternal aggression, some differences exist in the levels of aggression in the course of gestation and lactation (Olivier and Mos, 1986b).

2.2.5. Field of Application

The maternal aggression paradigm is mainly used to study the mechanisms of aggressive behavior in females. In particular the role of gonadal steroids has received much attention (Svare and Mann, 1983; Mayer and Rosenblatt, 1987). Recently, the paradigm has also been used in psychopharmacology (Olivier and Mos, 1986b).

2.3. Predatory Aggression

Predatory aggression can be defined as the type of attack that is evoked by the presence of a natural object of prey. It can be observed in all carnivorous animal species. However, also noncarnivorous species can perform predatory behavior. It can be demonstrated in rats killing mice or frogs (Karli, 1956; Bandler and Moyer, 1970) or in mice killing cockroaches (Brain and Al-Maliki, 1978). Ever since its discovery, the question of whether prey killing can be considered as a form of aggression has been heavily discussed (O'Boyle, 1974; van Hemel, 1975). A number of studies indicate that predatory aggression is very different from intermale fighting, not only in the structure of the behavior (Lynds, 1980), but also in the physiological neuroendocrine and genetic mechanisms involved (Brain and Al-Maliki, 1978; Ebert and Green, 1984). One should be extremely cautious, therefore, with extrapolations between predatory behavior and other forms of aggressive behavior.

The experimental procedure for eliciting predatory behavior is very simple. In the case of predatory behavior in the mouse, adult mice of either sex can be used. Each mouse is tested individually in its home cage. The test starts with introducing a suitable prey into the cage of the experimental animal. Behavior can be recorded by direct observation, during which a variety of behaviors can be distinguished (Brain and Al-Maliki, 1978). However, because it may take a long time before actual killing is observed, mostly killing latency and the number of animals showing a killing response are used. These parameters can be measured by routine checks at regular intervals.

2.3.1. Sex

There are no indications that sex is an important variable in predatory aggression (Butler, 1973).

2.3.2. Species/Strain

It is obvious that predatory behavior can be most reliably observed in carnivorous species, such as the cat. In other species, such as rodents, the proportion of animals that show predatory behavior is relatively small and varies between strains. About 20–30% of the laboratory rats spontaneously kill mice, whereas approx 70% of wild rats kill in the laboratory (Karli, 1956). In mice, about 10–30% of both males and females spontaneously kill locusts (Brain and Al-Maliki, 1978).

2.3.3. Food Regimen

Predatory behavior may be considered not only as a form of attack behavior, but also as a form of feeding. Indeed, food deprivation significantly increases the proportion of mice killing and consuming insects (Brain and Al-Maliki, 1978). Similar observations are reported on mouse killing in rats (Paul et al., 1971), in which rats are usually maintained on a 1 h/d feeding schedule.

2.3.4. Field of Application

Originally, the predatory aggression paradigm was used as a model of aggressive behavior. However, later studies showed that the mechanisms underlying predatory behavior were very different from those of intermale fighting or maternal aggression. Because of this, and the mixed motivational background, it is better to consider the predatory behavior as a behavior system in itself. It remains an open question regarding the extent to which it models human aggression.

2.4. Shock-Elicited Aggression

This paradigm is based on the hypothesis that pain is an important causal factor in aggressive behavior (Ulrich, 1966). The delivery of pain may result in biting by the subject on a conspecific or another suitable object (Azrin et al., 1964; Ulrich and Azrin, 1962).

Two tasks are commonly used to measure shock-elicited aggression. The most widely used task is the reflexive fighting test, in which two male rats are placed in a grid-floored chamber of about 25 × 25 × 30 cm. The grid floor can be electrified with a scrambled shock. Shock intensity may vary from 1.5–3 mA, and shock durations of 0.5–5 s are reported. Usually, shocks are repeatedly delivered with intervals of 1–15 s. No systematic study is available regarding these stimulus parameters. During the shock, the two animals may adopt a boxing posture, in which both forepaws are lifted off the grid floor. When sufficiently confined, biting may occur.

A second situation in which pain-elicited aggression is measured involves a shock or another sharp, sudden pain delivered to the tail of a rat, which is confined in a tube with a wooden dowel in front of its head for biting (Azrin et al., 1964). In this situation, the rat will bite during virtually every shock.

The value of this paradigm as a model of human aggression has been widely discussed. In an extensive analysis of both shock-induced aggression and intermale aggression in the resident intruder paradigm, Blanchard et al. (1977a) concluded that the behavior observed in response to a painful stimulus was very similar to the defensive behavior observed when a male rat was attacked by a dominant one. It was suggested that fear was the common denominator (Blanchard, 1984). This lack of specificity fits to the fact that also other types of behavior can be induced by a painful stimulus. For example, Caggiula and Eibergen (1969) observed copulatory behavior by applying painful electric shock to the tail of rats in the presence of an estrous female. In a subsequent study, Caggiula (1972) concluded that either sexual behavior or aggression could be elicited by manipulating stimulus parameters, such as shock frequency or environmental variables like cage size and gender of the stimulus animal. These experiments indicate that painful shock does not specifically induce aggressive behavior. It rather seems to induce fear and arousal. Several lines of evidence suggest that the behavioral response induced by this fear or arousal can be considered as a form of coping behavior. For example, paired rats that can fight when shocked show less pituitary adrenocortical activation—a presumed sign of stress—than when they receive the same foot shock alone (Conner et al., 1971). Few systematic studies are available on the parameters that are important in this model.

2.4.1. Age

Shock-induced fighting can be elicited after the first month of life, and the probability of the behavior increases over the course of months, reaching almost 100% at the age of 3 mo. However, a maximum probability of shock-induced fighting was reached at the age of 2 mo, when the animals were repeatedly tested for aggression from weaning onwards (Hutchinson et al., 1965).

2.4.2. Field of Application

The paradigm is mainly used in pharmacological studies and experiments aimed at the central nervous organization of this type of behavior.

2.5. Brain Stimulation Elicited Aggression

Electrical stimulation of the brain has been widely used to elicit behavioral responses. Hypothalamic areas have been extensively explored since the original report by Hess and Brügger (1943) on the role of subcortical structures in affective attack in the cat. Aggressive behavior can be induced by electrical stimulation of specific sites in the hypothalamus in a number of animal species, including the rat (Koolhaas, 1978; Kruk et al., 1983), the cat (Siegel and Edinger, 1983), and the monkey (Lipp and Hunsperger, 1978). In the cat and the rat, different forms of aggression can be elicited. In both species, intraspecific aggressive, offensive behavior can be elicited, a type of aggression that is known as affective attack in the cat (Kruk et al., 1979; Flynn, 1976). A second type of aggression that can be elicited is predatory behavior, i.e., quiet biting attack in the cat (Flynn, 1976) and mouse killing in the rat (King and Hoebel, 1968). Both the offensive behavior and the predatory behavior elicited by hypothalamic stimulation seem to be rather similar in form to the spontaneous behavior observed under nonstimulation conditions. For this reason, brain-stimulation-induced aggression is sometimes used as a model in which aggression can be elicited in a standardized way.

Basically, the experimental animals are implanted with bipolar electrodes, stereotaxically aimed at specific sites in the hypothalamus. The electrodes are permanently secured to the skull with screws and dental cement. A sine wave current or rectangular biphasic current pulse trains are used to stimulate the neural tissue. For a systematic study of the various stimulus parameters, such as current intensity, pulse frequency, phase duration, and train duration, the reader is referred to Kruk et al. (1983). The animals are usually tested in a kind of resident–intruder paradigm, in which the experimental animal is stimulated at regular intervals in the presence of a male conspecific of a slightly lower body weight. Under these conditions, aggressive behavior can be elicited reliably and in a highly stereotyped way. The behavior is little affected by environmental variables, such as type of opponent. The most important variable in this paradigm is the exact location of the electrode. An extensive overview of hypothalamic substrates for different behaviors, including aggressive behavior in the rat, is given by Lammers et al., 1988.

2.5.1. Field of Application

This paradigm is mostly used in the study of the central nervous organization of aggressive behavior in a number of animal species. In the rat, the paradigm has also been used for pharmacological studies. It appears that hypothalamic stimulation-induced aggression does not react to most of the drugs that are effective in spontaneous aggression (Kruk et al., 1987), with the exception of serotonergic acting drugs.

2.6. Genetic Models

A considerable amount of evidence indicates that variations in aggressive behavior may be attributed to genetic predispositions (Fuller and Thompson, 1978). Although differences between various strains of animals in several forms of aggressive behavior are well known, only a few true genetic selection lines are available. Selection for high and low levels of aggressive behavior has mainly been performed in mice.

For example, van Oortmerssen and Bakker (1981) selected male wild house mice for short and long attack latencies in a resident–intruder paradigm. The resulting short attack latency line and long attack latency line appeared to differ not only in their level of aggressive behavior, but more generally in active and passive coping with environmental challenges (*see* Section 1.1., and Benus et al., 1989, 1991). Almost similar conclusions were obtained on the basis of selection experiments in mice by Gariépy et al., (1988). Using a rather complex selection criterion, Ebert and Hyde (1976) obtained genetic selection lines for high and low levels of female aggression. Surprisingly little attention has been paid to these genetic models in relation to the pathology of aggressive behavior, in particular, because recent studies indicate that high levels of aggressive behavior are maladaptive in the population dynamics of wild house mice (van Oortmerssen and Busser, 1989).

3. Concluding Remarks

Most of the animal models of aggression presented in this chapter are aimed at understanding normal, i.e., nonpathological, forms of aggression. Most models are behavioral similarity models and mechanistic models of human nonpathological aggression. With respect to the validity of the various models, the resident–intruder paradigm and the maternal aggression model seem to be on face value the most realistic models for male and female aggression, respectively, in humans. The large amount of literature on the neuroendocrine, central nervous, and neuropharmacological aspects of aggression in these models suggests a considerable predictive—and construct—validity as well. The validity of the various animal models to human aggression and violence in psychiatry is still speculative, however. One of the most extensively studied syndromes that has been associated with human violence is episodic dyscontrol or explosive rage. This syndrome is frequently attributed to seizure activity in the temporal lobe (Monroe, 1985; Gedye, 1989). Surprisingly few animal studies pay attention to this syndrome. Blanchard and Blanchard (1980) argue that a substantial set of dyscontrol patients display an alteration in fear-based attack tendencies, which may be analogous to defensive aggression observed in the intruder male in the resident–intruder paradigm or in the pain-elicited aggression model. However, their neurological evidence is mainly based on reduced defensive behavior after amygdaloid or striatal lesions, rather than increased defensive attack resulting from excitation of limbic brain structures.

Kindling procedures to produce epileptic activity in specific brain structures (Goddard et al., 1969) have rarely been used to study the role of focal epileptic activity in pathological forms of aggressive behavior. Pinel et al. (1977) found an increased reactivity and resistance to capture in rats with experimentally induced epileptic activity in the amygdala or hippocampus, but not in the caudate nucleus. The authors interpret this as an increase in aggression. However, in a more extensive study using a variety of aggression models, Bawden and Racine (1979) could not confirm this phenomenon. Some recent experiments in our laboratory suggest, however, that amygdala kindling does change intraspecific aggressive behavior in a resident–intruder paradigm. Although the data are still preliminary, they show that the role of epileptic activity in aggressive behavior needs more experimentation. Experiments using electrical and neurochemical stimulation of brain structures seem to be promising as well in this regard. For example, Bandler et al. (1980) found that hypothalamic and lower brain stem stimulation, eliciting predatory attack in the cat, changes patterns of forebrain activity, which may be relevant to patterns of neuronal dysfunctioning underlying the dyscontrol syndrome.

The object of this chapter has been to review various animal models frequently used in aggression research. Animal models serve an important role for research of human aggression, because they provide information regarding the mechanisms that may affect the probability for the occurrence of aggressive behavior. Although few animal studies are directly aimed at understanding human violence, insight into the basic mechanisms of aggressive behavior is fundamental in understanding its pathology.

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Animal Models of Mental Retardation

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1. Introduction

Children with mental retardation typically show a general developmental delay, whereas in specific developmental disorders, there is a delay in a specific area of behavior, such as reading or speech. According to DSMIII (American Psychiatric Association, 1980, 1987), the diagnostic criteria for mental retardation include:

1. Significantly subaverage general intellectual functioning, i.e., an IQ of 70 or below on an individually administered IQ test.
2. Concurrent deficits or impairments in adaptive functioning, i.e., an individual's effectiveness in meeting standard expectations in a range of areas, such as social skills and responsibility, communication, daily coping, personal independence, and self-sufficiency.
3. An onset before the age of 18, i.e., the mental retardation is manifested during the developmental period.

Thus, the degree of mental retardation has been classified on a system based almost exclusively on IQ levels (McCandless, 1964), and includes *mild* (IQ 55–70), *moderate* (40–54), *severe* (25–39), and *profound* (IQ below 25) retardation. Of the cases of mental retardation with a known organic cause, most fall into the severe or profound category, and require institutionalized care. The organic causative factors vary from genetic abnormalities (e.g., Down's syndrome) to maternal infections or pregnancy disorders (e.g., rubella or drug ingestion), from birth complica-

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tions (e.g., oxygen deficit) to postnatal factors, such as infections (e.g., encephalitis or meningitis), head injuries, or neurotoxic poisoning.

It has been suggested elsewhere that the neurochemical/neuroanatomical basis of general learning deficits was a primary requirement for studying the processes governing brain abnormalities in mental retardation (Archer, 1987). In hindsight, this appears to be an oversimplification, since mental retardation appears to be caused by a multitude of known and unknown factors, but generally the etiology of these cases cannot be or is difficult to identify. One general strategy is to develop a better understanding of the neural mechanisms of learning and memory, and through the "processes of serendipity," achieve sufficient knowledge of brain structures and genetic materials to predict certain causal relationships.

In order to describe animal models of mental retardation, it is necessary to stipulate the reliable and robust demonstration of a generalized performance disruption in a variety of learning tasks that may be accompanied by other behavioral changes, e.g., hyperactivity and/or reduced exploratory behavior. Learning procedures for testing retardation ought to encompass as wide a spectrum of the standard procedures as possible, such as maze learning, classical conditioning phenomena with compound stimuli, and exploration in diverse situations. For example, Thompson and his coworkers (1987) have outlined a test battery incorporating both aversively and appetitively motivated tasks: visual discrimination, vestibular-proprioceptive-kinesthetic discrimination, an enclosed three-cell maze, or mounting a raised platform, entering an elevated cylinder, and climbing a ladder to obtain a goal object. On the other hand, many investigators have applied such maze learning tests as the Morris swim maze, the Olton radial arm maze, and the Hebb-Williams problem-solving maze.

In this review, we shall describe several potential animal models of mental retardation, including:

1. Nonselective neurotoxic treatment of the developing fetus;
2. Selective neurotoxin-induced models of mental retardation;
3. Congenital models of mental retardation;

4. Brain lesions early in development;
5. Age-related changes (since these in some respects parallel certain ontogenetic alterations);
6. Differential housing environment during development;
7. Interactions of differential rearing conditions with neurochemical lesions; and
8. Teratological effects related to mental retardation (using prenatal organic tin and alcohol effects as an illustration).

2. Nonselective Neurotoxic Treatment of the Developing Fetus

Following treatment to dams at either day 14 or 15 of gestation, the antimitotic agent methylazoxymethanol (MAM, 25 mg/kg, sc) causes offspring with marked forebrain microencephaly (Fisher et al., 1972; Spatz and Laquer, 1968), a markedly retarded condition clearly corresponding to the general requirement of a neuropathological state. MAM-treated offspring show a diverse range of neurochemical and neuromorphological changes (Johnstone and Coyle, 1979,1980; Jonsson and Hallman, 1982). Extensive biochemical analyses have been described by Hallman (1984). Monoamine concentrations of MAM and control rats in various brain regions are indicated below (*see* Table 1). These neurological changes, which suggest a relative hyperinnervation of forebrain monoamine terminals, have been correlated with severe and consistent impairments in the performance of both aversively and appetitively reinforced learning tasks (Archer et al., 1988b; Haddad et al., 1969; Vorhees et al., 1984; Mohammed et al., 1986b,c).

MAM-treated rats display a characteristic hyperactivity in several test situations, but generally more so in familiar environments. Archer et al. (1988a,b) obtained notable increases in several parameters of spontaneous and stimulant drug-induced motor activity, as measured by direct observation or in automated photocell test cages. This hyperactive state was accompanied by notable impairments in the acquisition of spatial instrumental learning tasks, i.e., the Morris swim maze and the Olton radial arm maze. Figure 1 presents results indicating the performance of MAM and control rats in the swim maze task

Table 1
Effect of Prenatal Methylazoxymethanol Treatment
on Monoamine and Metabolite Concentrations in Brain Regions[a]

		ng/g Wet weight of the tissue[b]		
		Frontal cortex	Striatum	Hippocampus
DA	Control	34.1 ± 3.2**	12955 ± 801**	58.9 ± 17.8
	MAM	150.7 ± 12.9	23368 ± 933	68.7 ± 6.2
	(%)[c]	(442)	(180)	(117)
NA	Control	488.1 ± 23**	—	575.5 ± 58.6*
	MAM	1217.9 ± 21	—	891.5 ± 14.4
	(%)	(250)		(155)
DOPAC	Control	12.6 ± 1.3**	2440 ± 216*	32.5 ± 8.6
	MAM	80.3 ± 18.3	3791 ± 291	44.9 ± 5.1
	(%)	(637)	(155)	(138)
HVA	Control	11.8 ± 3.2**	949 ± 141*	38.2 ± 5.1
	MAM	63.0 ± 15.8	1441 ± 227	49.1 ± 8.1
	(%)	(534)	(152)	(129)
5-HT	Control	516.3 ± 25.3**	405.0 ± 34.0**	408.3± 12.6**
	MAM	1045.4 ± 61.8	1087.6 ± 99.8	737.4 ± 23.0
	(%)	(203)	(269)	(181)
5-HIAA	Control	253.5 ± 15.5**	426.3 ± 42.7*	554.1 ± 32.6*
	MAM	519 ± 66.6	891.2 ± 99.3	765.1 ± 65.0
	(%)	(205)	(209)	(138)

[a] Methylazoxymethanol was administered to pregnant dams (25 mg/kg, sc) on gestation day 15 (*see* Jonsson and Hallman, 1982).

[b] The values are expressed as mean ± SEM.

[c] (%) = percent of control values.

** $p < 0.001$, * $p < 0.01$, Student's *t*-test.

and in the radial arm maze. Surprisingly, MAM offspring did not differ from saline-treated offspring in the acquisition of the difficult differential reinforcement of low rates 72 second task, although they did make fewer responses on a Fixed Ratio 1 schedule. Finally, MAM offspring consistently failed to show both context-dependent and context-independent latent inhibition effects (Mohammed et al., 1986a,b).

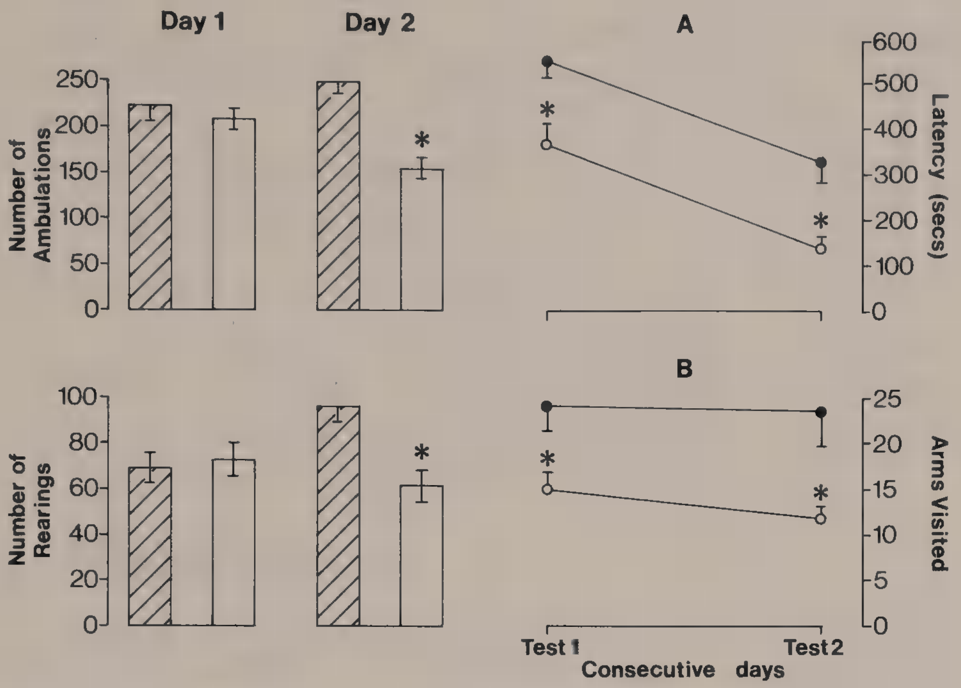

Fig. 1. Spontaneous motor activity over a 15-min test period in a modified Olton maze as indexed by the number of ambulations and rearings by MAM-treated (●—●) and control (○—○) rats at an age of about 11 wk (left-hand panel). Radial arm maze learning performance (i.e., the latency to acquire all eight pellets distributed one to each of the eight arms and the number of arms visited in doing so) by MAM-treated and control rats about 1 wk after the motor activity test (apparatus and procedure are described in Archer et al., 1988b,c).

Nonselective damage to brain tissues resulting from recurrent convulsive seizures following kainic acid treatment (Lothman and Collins, 1981; O'Shaughnessy and Gerber, 1986; Sperk et al., 1983) may also constitute a model of mental retardation. Milgram and coworkers (Milgram et al., 1988, submitted) have studied extensively the effects of systemic kainic acid treatment (10 mg/kg) upon spontaneous motor activity, passive avoidance using light–dark compartments, and spatial learning in a circular water maze (Morris-type). Rats treated with kainic acid showed hyperactivity in the open-field test, shorter latencies to reenter the dark compartment after having been shocked there, and considerably longer escape latencies in the water than the control animals (Milgram et al., 1988; *see* Fig. 2). Systemic kainic acid treatment produces extensive damage to the brain, but there

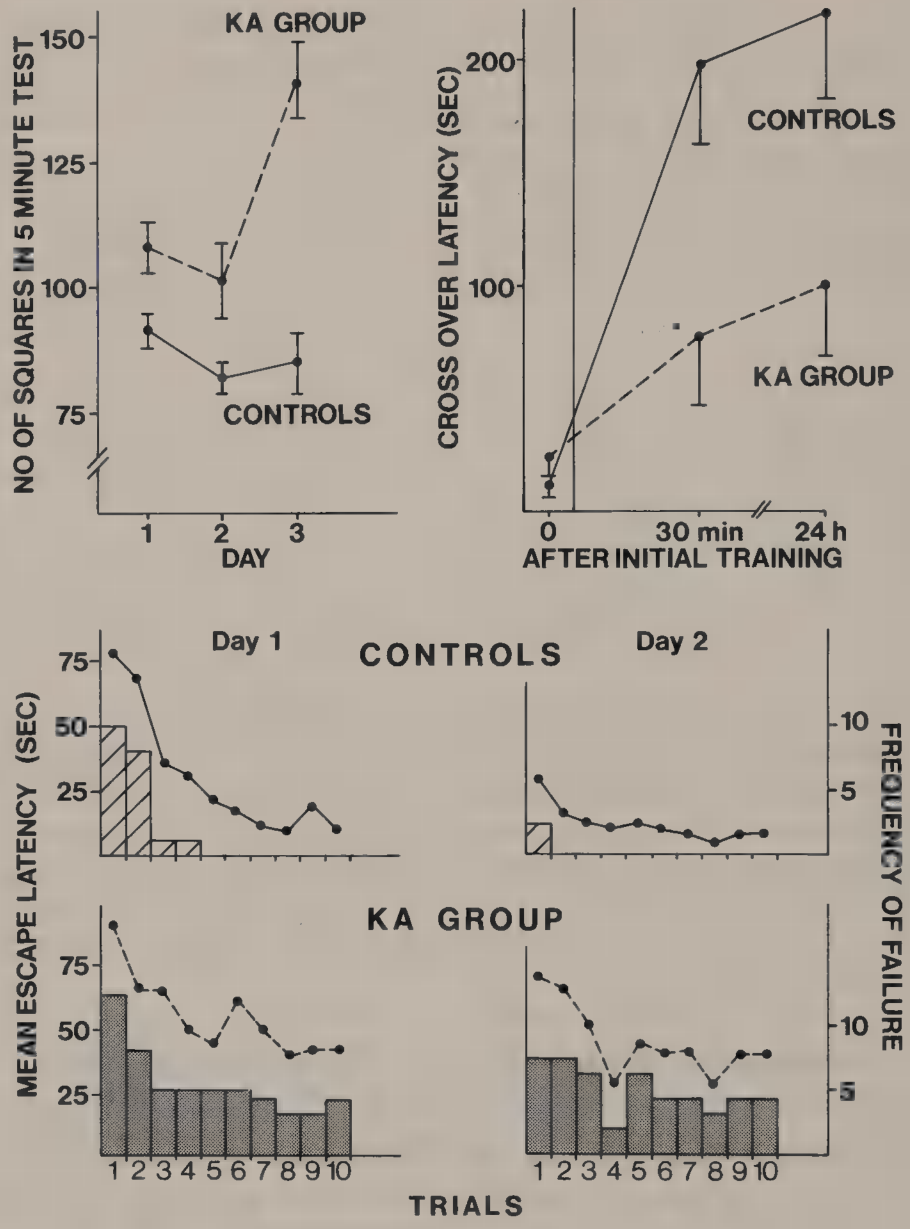

Fig. 2. Top panel: scores on open-field activity tests over three successive daily sessions (+ SE). Bottom panel: Spatial learning in a circular water maze for kainic acid and control groups. Curves represent mean latencies to locate and climb onto the submerged platform. Histograms represent the number of animals in each group failing to locate the platform during the 90-s tests. (Data redrawn from Milgram et al., 1988.)

appears to be a degree of selectivity in that only particular brain structures and only certain cell types within those structures are affected (Nadler and Cuthbertson, 1980). In the context of a retardation model, it should be noted that a similar neuropathology is produced by a wide range of seizure-inducing chemicals with structures and pharmacological profiles different from that of kainic acid (Honchar et al., 1983; Olney et al., 1982).

3. Selective Neurotoxin-Induced Models of Mental Retardation

Several more or less selective neurotoxins are available for use in functional investigations, although it is doubtful whether most are suitable for obtaining mental retardation models. Notwithstanding this reservation, the selective neurotoxins do allow pharmacologically relevant models.

3.1. Cholinergic Systems

The ethylcholine mustard aziridinium ion, AF64A, related in structure to choline, has been studied quite extensively (Fisher and Hanin, 1980; Fisher et al., 1982; Mantione et al., 1981). For instance, intracerebroventricular (icv) injections of AF64A (65 nmol) in mice caused significant decreases in acetylcholine levels, high-affinity choline transport, and choline acetyltransferase activity in the cerebral cortex, hippocampus, and striatum for at least 7 d after treatment. Other reports suggest long-term learning deficits, including radial arm maze performance and passive avoidance retention, up to 5 wk after icv injections to rats (Walsh et al., 1983). In spite of these interesting observations, other evidence suggests the incidence of notable nonspecific damage by this neurotoxin, the status of which is still open to question (cf Jarrard et al., 1984).

3.2. Serotonergic Systems

Several relatively selective serotonergic neurotoxins have been applied to study effects upon the performance of a wide variety of learning tasks. These include 5,6-dihydroxytryptamine, 5,7-dihydroxytryptamine, and even *p*-chloroamphetamine at high doses administered systemically. Although it is quite likely that serotonin depletions do affect some forms of avoidance learning (e.g., Lorens, 1973), the exact significance of these diverse effects with regard to a mental retardation model is difficult to assess.

3.3. Catecholaminergic Systems

6-Hydroxydopamine (6-OHDA) may be used to deplete either dopaminergic or noradrenergic terminals, depending upon the brain region to which it is applied, and the particular uptake inhibition used for pretreatment to protect either dopamine (DA) or noradrenaline (NA). Generally, selective NA depletions can be obtained through microinjections of 6-OHDA into the dorsal noradrenergic bundle or by systemic injections of the NA neurotoxin, DSP4. It seems fair to say that this treatment does not result in a "general learning" deficit, but rather alterations in compound classical conditioning phenomena, such as sensory preconditioning, latent inhibition, and blocking (Archer et al., 1983, 1986; Mohammed et al., 1984). However, it has been claimed that selective NA lesions have led to impairments in discrimination and T-maze alternation tasks (Mason and Iversen, 1977, 1978; Mason et al., 1983), although this effect has not always been easy to replicate (e.g., Hagan et al., 1983; Pisa and Fibiger, 1983).

More interesting as a possible model for mental retardation is the administration of 6-OHDA (either intracerebroventricular, icv or intracisternal, ic) to newborn rats once or twice from day 1 to day 6 after birth, and at doses that range from 50 μg to 2 × 100 μg. The behavioral alterations following DA depletion after neonatal 6-OHDA treatment appear to consist of:

1. A marked degree of hyperactivity, which may or may not persist well into adulthood (Erinoff et al., 1979; Miller et al., 1981; Shaywitz et al., 1984);
2. Deficits in the performance of shock-avoidance tasks (Shaywitz and Pearson, 1978; Smith et al., 1973);
3. A retarded acquisition of lever-pressing responses on a fixed-ratio schedule of water reinforcement (Heffner and Seiden, 1983); and
4. Impaired and/or retarded performance of instrumental maze learning tasks (Archer et al., 1988c; Archer et al., submitted; Oke and Adams, 1978; Wishaw et al., 1987).

Neonatal DA depletion induced quite complex alterations of motor activity. For example, DA-depleted rats, studied by direct observation using video apparatus in a modified radial arm maze, showed much less rearing activity than control rats during the 10-min tests and also during the initial 20-min period in automated photocell cages. During the second half of 60-min test periods, the 6-OHDA-treated rats showed drastic hyperactivity (Archer et al., 1988c). We have obtained data in open-field-holeboard experiments (*see* Fig. 3aC and bC). Thus, DA-depleted rats showed more locomotor activity, but less rearing and head-dipping activity, which has led us to suggest that the DA-depletion-induced hyperactive condition is accompanied by some loss of effective exploratory behavior.

Figure 3 summarizes the data from two experiments in which newborn rats received 2 × 100 μg icv 6-OHDA. Acquisition performance for spatial learning tasks was tested in the Morris maze and in the radial arm maze. The results from experiments using 6-OHDA to deplete DA neonatally suggest that this technique may offer a pharmacologically selective animal model of retardation. Preliminary results (Mohammed et al., submitted; Archer and Mohammed, 1989) suggest that differential housing following weaning may modulate the neurotransmitter-related changes, i.e., enriched housing decreased the hyperactivity and improved maze learning performance of DA-depleted rats to control levels *(see below).*

4. Congenital Models of Mental Retardation

Certain congenital models of mental retardation may be considered, possibly in view of the relative validity that may be offered in particular instances. Thus, a growing number of physiological and behavioral investigations have been performed on mutant strains of rats and mice, and these may well constitute potentially viable models: albino and hairless (Mount, 1971), dwarf (Snell, 1929; Bouchon, 1984), reeler (Falconer, 1951; Caviness, 1980), staggerer (Sidman, 1968; Ando and Matsui, 1986), shaker short-tail (Lyons and Wahlsten, 1988), and weaver (Bullock et al., 1982). In the present analysis, three of these mutant strains, dwarf, staggerer, and reeler, will be considered.

Dwarfism in mice appears to correspond not only to the syndrome of human congenital hypothyroidism, but also to the relatively rare antero-hypophyseal deficiency (Delange et al., 1977;

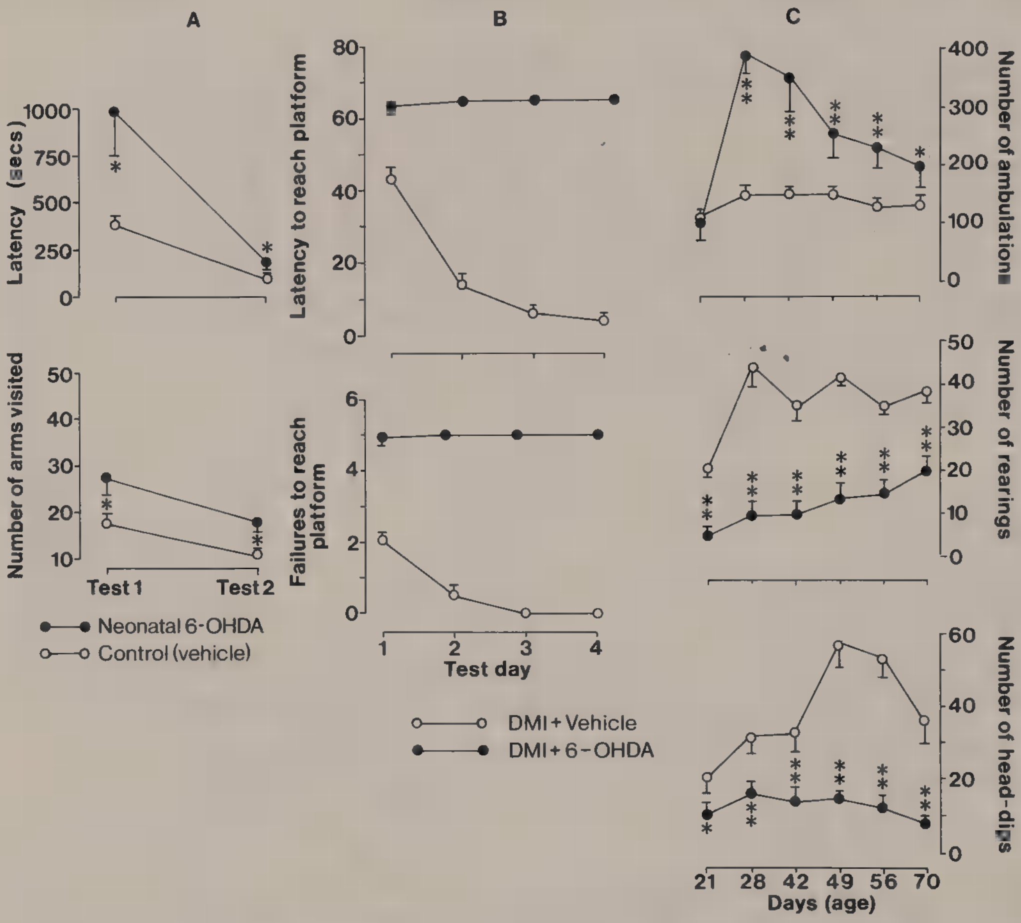

Fig. 3A. Mean latency to take all eight food pellets and mean number of arms visited in a radial eight-arm maze by neonatally 6-OHDA-treated and vehicle-treated rats (A). Mean latency to locate the platform and number of failures to locate it in a Morris-type swim maze (B). Mean number of ambulatory, rearing, and head-dipping behaviors in a modified open-field holeboard by neonatally 6-OHDA-treated and vehicle-treated rats (C) (icv 200 µg, 30 min after desipramine, 25 mg/kg; apparatus and procedure described in Archer et al., 1988c).

Fisher, 1975; Klein et al., 1975; MacFaul and Grant, 1977) characterized by cretinism and hypoactivity. Dwarf mice have been found to show both hypoactivity (Bartke, 1964) and deficits in maze-learning performance (Bouchon and Will, 1982a). The neuroanatomical, endocrine, and neurochemical changes include an almost total absence of somatotrophin and thyroid-stimulating hormones (Carsner and Rennels, 1960; Elftman and Wegelius 1959; Wegelius 1959), as well as a retarded maturation in cerebellar and lumbosacral regions (Reier et al., 1972,1974,1975).

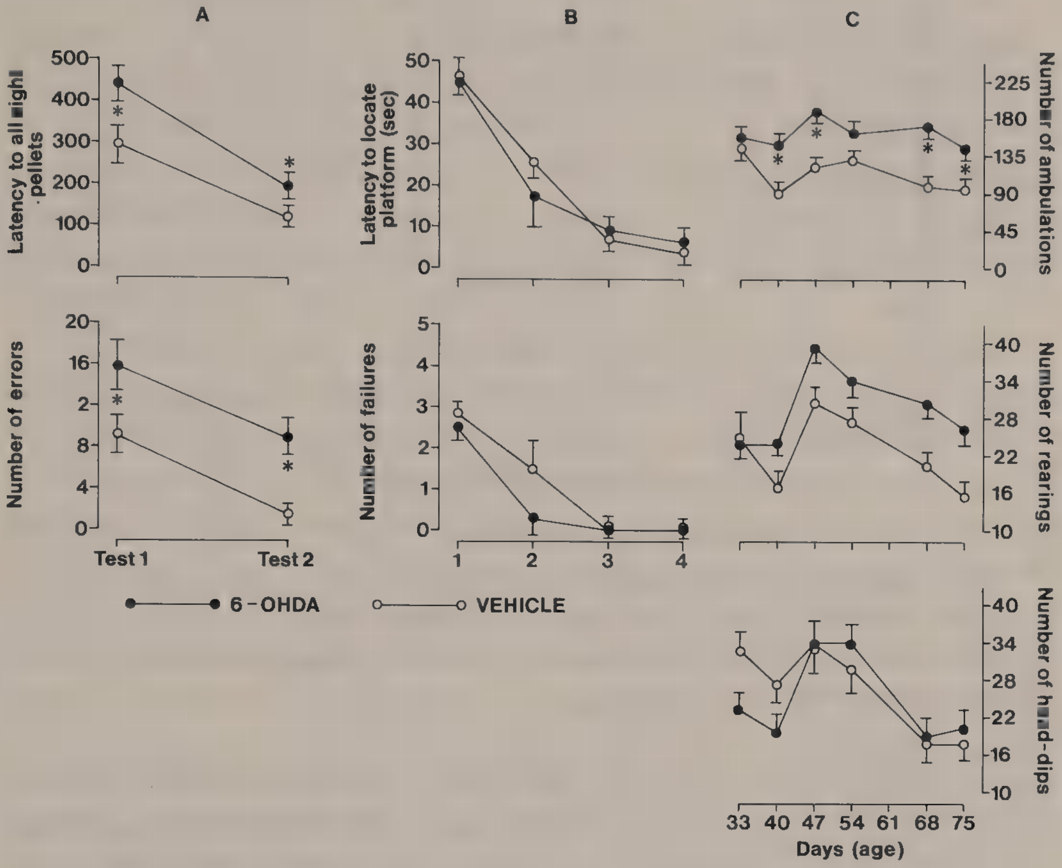

Fig. 3B. Mean latency to take all eight food pellets and mean number of arms visited in the radial arm maze by neonatally 6-OHDA-treated and vehicle-treated rats (A). Mean latency to locate the platform and the number of failures to locate it in a Morris-type swim maze (B). Mean number of ambulatory, rearing and head-dipping behaviors in a modified open-field holeboard by neonatally 6-OHDA-treated and vehicle-treated rats (ic 50 µg, 30 min after desipramine, 25 mg/kg; apparatus and procedure described in Archer et al., 1988c).

Bouchon and Will (1982b) have found reduced exploratory behavior, and a failure to show habituation in an open field and in automated test cages for activity (*see below* and Fig. 4).

The *staggerer* mutant strain, resulting from a single recessive gene, is characterized by a severe reduction in cerebellum size owing to a synaptogenesis failure between Purkinje cells and parallel fibers. Cerebellar abnormalities cause drastically altered gait and posture. Locomotion is poorly coordinated, mainly a shuffling/lurching motion from side to side together with loss of balance. Most of the behavioral disturbances appear

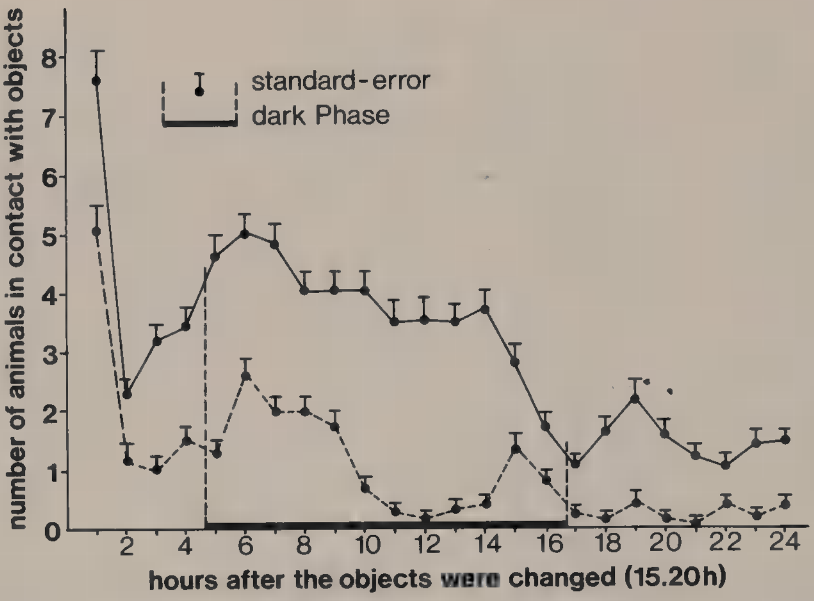

Fig. 4. Mean number of "dwarf" (—; $N = 12$) and control (—; $N = 12$) mice in contact with the objects of the enriched environment at each hour of a 24-h cycle (mean + SE). Means over a 31-d observation period. Data redrawn from Bouchon and Will (1982b).

to be directly related to the cerebellar deficiencies. Exploratory behavior may be affected under some circumstances. Goodall (1985) observed that, in a terrestrial maze, staggerer mice remained within a restricted area close to the entrance of the maze, whereas normal mice explored all the cells of the maze. However, other investigators, using different apparatus and procedures, appeared not to confirm the alterations in exploration (e.g., Lalonde et al., 1987; Misslin et al., 1986). It seems clear that staggerers showed a contrastingly different pattern of exploration in novel environments (Guastavino, 1978; Goodall and Guastavino, 1986; Hamidon and Guastavino, 1986). It is probable that at least part of the alteration in exploratory behavior is a result of some gross deficiency in the neophobia and/or defensive behavior of staggerer mice (Misslin et al., 1986).

Staggerer mutant mice also show abnormalities in other types of behavior, such as swimming, sexual behavior, and maternal behavior. Goodall et al. (1986) used a swim apparatus consisting of a glass box, 1 m^2 and filled to a depth of 10 cm with tapwater, to study swimming behavior with rather more perseverative tendencies than normal. Although Sidman et al.

(1962) reported that staggerer mice were unable to breed, Guastavino (1982) has subsequently shown that male staggerer mice could copulate with normal female mice and vice versa. There is no doubt, however, that sexual activity is greatly affected, and this disorder is not solely the result of the cerebellar abnormalities (Guastavino, et al. in press). Staggerer mouse dams seem incapable of fostering their young unless adequate precautions are taken (Guastavino, 1983, 1984).

The *reeler* mutant mouse strain, also resulting from a single recessive gene, is characterized by a disarray of laminae in the cerebellar and cerebral cortex, as well as the hippocampus (Meier and Hoag, 1962). Synaptogenesis and function of the affected neurons appear unaltered, although structural localization is abnormal. One distinguishing behavioral characteristic is hypoactivity, but this seems to vary with individual mice and degree of novelty of the test environment (Guastavino et al., in press; Myers, 1970). As with the staggerer mouse strain, there are gross deficiencies in the ability of male and female reeler mice to display sexual behavior (Allain, 1983; Myers, 1970). The same authors have also shown that female reeler mice were only partially able to display adequate nest-building behavior, as well as suckling, grooming, and retrieving of pups.

5. Interactions of Mutant Mouse Strains with Differential Rearing Conditions

Guastavino (1988) has shown that certain behavioral deficiencies, e.g., abnormal gait, may be improved in staggerer mice given daily access to environmental stimulation. The improvement, however, did not appear to lead to a restoration. Bouchon and Will (1982b, 1983) have investigated the effects of differential rearing environments (enriched vs restricted) on locomotor activity and exploratory behavior, as well as on spontaneous alternation in dwarf mice. It appears that no significant improvements in the behavioral deficiencies of dwarf mice are produced by enriched housing, although this is certainly the case with staggerer mice. Allain (1983) found that environmental stimulation improved the motor ability of the reeler mutant strain.

6. Brain Lesions Early in Development

One of the most ambitious attempts to create an animal model of mental retardation is the work of Thompson and coworkers (e.g., Thompson et al., 1986, 1989a,b). In their work, groups of weanling rats (21–24 d old) received lesions in a large number of cortical and subcortical brain areas, the sites selected forming part of the major subdivisions of the brain, such as the neocortex, basal ganglia, limbic system, thalamus, hypothalamus, and brainstem. After a recovery period of at least 3 wk, the rats were subjected to a test battery consisting of several separate learning tasks, including detour problems, a 3-cul maze, visual and nonvisual discrimination habits, and motor-skill tasks.

A brain-lesioned rat is regarded as being retarded if it is deficient on all of these tasks, but one of the most sensitive measures of this global cognitive deficit is the detour problems (Thompson et al., 1989a). The detour apparatus, shown in Fig. 5, is divided into a startbox, a choice chamber, and a goalbox. The partition between the choice chamber and the goalbox is interchangeable, so that the thirsty rat in order to reach the goalbox must either mount the raised platform (top panel), enter the elevated cylinder (middle panel), or climb the ladder (lower panel).

Using the detour problems and the other cognitive tasks, some 25 separate groups of lesioned rats were assessed for the occurrence of a pervasive learning deficit, i.e., an increased number of errors relative to the controls, on most, if not all, of the learning problems (Thompson et al., 1986). As shown in Table 2, focal damage to the parietal cortex, destroying about a fifth of the total neocortex, produced significant learning deficits on most of the problems. This global learning impairment was not present in the other groups with focal cortical lesions, though it should be noted that diffuse cortical destruction (16 punctate lesions in various cortical regions destroying about one-third of the total neocortical mantle) also elicited global learning impairments.

As shown in Table 2, lesions to certain components of the basal ganglia also impaired learning ability. Thus, lesions situated in the caudate-putamen (striatum) and the substantia nigra caused more errors than controls on the various tasks, and it

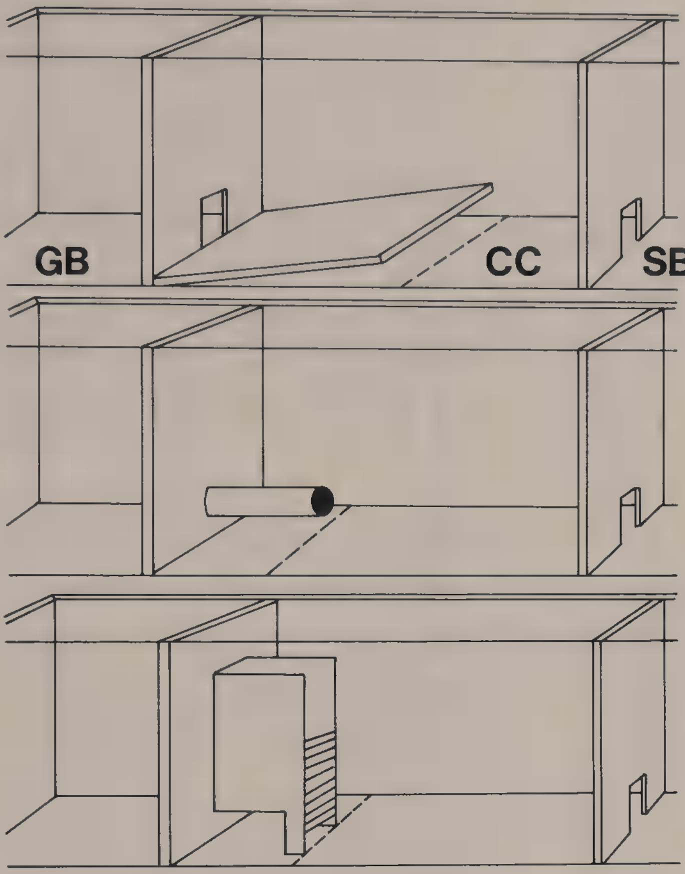

Fig. 5. Schematic drawing of the detour apparatus, showing the startbox (SB), choice chamber (CC), and goalbox (GB). The interrupted lines mark the boundaries of the blind alleys. On Problem A (top panel), the rat must mount the raised platform in order to gain access to the goalbox. On Problem B (middle panel), the rats must enter the elevated cylinder to reach the goalbox. On Problem C (lower panel), the rat must climb the ladder to reach the goalbox. (Diagrams redrawn from Thompson et al., 1989b.)

was also shown that destruction of the ventral tegmental area of Tsai (medial to the substantia nigra) produced similar deficits. Importantly, these basal ganglia learning deficits were not attributable to motor disorders.

Whereas none of the lesions placed in various portions of the limbic system produced global learning deficits, destruction of specific structures in the thalamus and the brainstem did so. Thus, as seen in Table 2, animals with lesions in the ventrolateral thalamus, median raphé, or pontine reticular formation were significantly less efficient than controls on all six problems; it

Table 2
Total Error (Mean and SE) Scores for All Groups on Each of the Six Problems Composing the Test Battery

		Aversively motivated problems				Appetitively motivated problems		
Group	N	Visual discrimination	Maze	Inclined plane discrimination	N	Detour A	Detour B	Detour C
Control	86	20.8 ± 0.5	10.0 ± 0.4	7.4 ± 0.3	60	5.8 ± 0.3	6.8 ± 0.8	5.0 ± 0.4
Neocortex								
Frontal	11	20.8 ± 2.6	7.5 ± 0.6	11.0 ± 1.3	11	14.5 ± 1.8*	10.6 ± 2.1*	12.7 ± 1.7
Parietal	10	36.0 ± 4.1*	30.9 ± 4.8*	18.9 ± 1.7	10	12.0 ± 1.7*	9.9 ± 2.2*	8.5 ± 1.6
Occipitotemporal	9	57.6 ± 1.5*	36.0 ± 7.5*	12.6 ± 2.1	10	5.6 ± 1.1	13.5 ± 1.5*	6.3 ± 1.3
Punctuate (12 lesions)	10	28.7 ± 2.0*	23.9 ± 3.4*	10.5 ± 1.1	8	12.0 ± 1.9*	8.1 ± 1.9	7.4 ± 1.1
Punctuate (16 lesions)	9	61.8 ± 4.2*	44.1 ± 9.0*	20.6 ± 3.9	7	16.7 ± 1.4*	18.6 ± 1.0*	13.1 ± 2.0
Basal ganglia								
Caudoputamen	13	21.7 ± 2.0	13.2 ± 1.1*	10.0 ± 1.0	13	11.1 ± 1.1*	10.7 ± 2.2	8.9 ± 1.2
Globus pallidus	7	43.0 ± 4.6*	16.3 ± 1.8*	13.3 ± 1.2	12	9.3 ± 1.0*	13.1 ± 2.0*	7.8 ± 1.3
Entopeduncular nucleus	12	27.6 ± 2.1*	16.2 ± 3.3*	8.7 ± 0.9	12	14.9 ± 1.4*	17.8 ± 1.1*	13.3 ± 1.6
Substantia nigra	8	31.3 ± 2.9*	15.5 ± 1.7*	19.9 ± 2.9*	9	11.9 ± 2.2*	17.7 ± 1.2*	13.6 ± 2.0
Limbic forebrain								
Frontocingulate cortex	9	17.1 ± 1.7	20.0 ± 3.3*	18.9 ± 2.9*	17	11.8 ± 1.6*	14.5 ± 1.5*	8.1 ± 1.5
Posterior cingulate	10	15.9 ± 1.9	31.0 ± 2.5*	19.2 ± 2.1*	13	13.9 ± 1.4*	10.5 ± 2.0	10.8 ± 1.6
Dorsal hippocampus	10	22.7 ± 2.1	33.6 ± 4.4*	11.8 ± 1.5	10	15.6 ± 1.9*	16.6 ± 2.2*	18.2 ± 0.8
Amygdala	7	18.3 ± 2.3	13.3 ± 1.8*	6.3 ± 0.8	7	5.9 ± 0.7	5.6 ± 1.6	4.7 ± 1.0

Thalamus								
Anterior complex	7	13.6 ± 1.9	33.9 ± 3.6*	18.1 ± 1.3*	7	16.9 ± 1.4*	14.3 ± 2.7*	16.3 ± 2.2
Lateral complex	7	23.7 ± 2.4	37.1 ± 3.5*	20.0 ± 2.2*	11	7.0 ± 0.8	10.5 ± 2.1	8.4 ± 1.7
Ventrolateral complex	8	27.5 ± 3.9*	17.6 ± 3.5*	24.5 ± 2.3*	8	11.1 ± 2.5*	12.0 ± 1.7*	10.9 ± 2.3
Mediodorsal complex	9	15.7 ± 2.1	20.3 ± 3.6*	12.8 ± 1.3*	9	15.0 ± 2.1 *	8.0 ± 2.1	9.8 ± 1.8
Parafascicular complex	9	20.0 ± 1.4	21.0 ± 4.1*	17.2 ± 2.1*	9	17.2 ± 1.2*	16.9 ± 1.2*	7.7 ± 1.8
Ventral complex	6	27.3 ± 2.7*	13.0 ± 0.6*	10.8 ± 1.8	7	5.6 ± 1.2	3.7 ± 0.9	4.4 ± 1.4
Hypothalamus (lateral)	10	29.8 ± 3.4*	39.1 ± 5.6*	12.2 ± 1.1	7	15.6 ± 2.3*	14.1 ± 2.0*	16.9 ± 2.0
Reticular formation								
Rostral midbrain area	9	17.5 ± 1.8	15.0 ± 1.6*	30.5 ± 2.9*	8	11.4 ± 2.2*	11.8 ± 2.0*	4.9 ± 2.1
Pontine area	6	47.7 ± 4.0*	38.7 ± 6.8*	26.8 ± 5.2*	13	10.3 ± 1.2*	8.6 ± 1.1*	6.0 ± 1.1
Other brainstem areas								
Midbrain central gray	9	29.0 ± 1.7*	15.8 ± 3.1 *	18.7 ± 2.0*	10	10.3 ± 1.4*	8.8 ± 1.8	6.1 ± 1.2
Median raphé	6	48.8 ± 4.0*	44.4 ± 9.0*	24.5 ± 4.4*	11	14.6 ± 1.9*	18.8 ± 0.8*	17.0 ± 1.4*
Lateral midbrain area	7	21.1 ± 1.5	7.0 ± 3.5	10.0 ± 0.6	11	5.5 ± 1.0	6.4 ± 1.8	5.6 ± 1.2

*Differs from respective sham-operated control subgroup, Mann-Whitney U, $p < 0.05$. Reproduced with permission from Thompson et al. (1986).

has later been discovered that lesions in the superior colliculus also impaired the acquisition of such learning problems.

In summary, it is clear that, when young rats receive lesions to certain parts of the brain—parietal cortex, striatum, globus pallidus, ventrolateral thalamus, substantia nigra, ventral tegmental area, superior colliculus, median raphé, or pontine reticular formation—they exhibit a global learning impairment in adulthood. This cognitive deficit cannot be explained on the basis of deficits in any one sensory modality or motivational state, and the impairments are seen in several classes of laboratory problems.

As pointed out by Thompson et al. (1989b), it is remarkable that the majority of the above-mentioned structures form part of, or are intimately connected with, the basal ganglia. It may be, therefore, that the basal ganglia and its associated brain regions form part of a General Learning System, consisting of a vertically organized anatomical system that extends from the telencephalon to the lower mesencephalic and pontine levels. In the normal animal, this system is seen as playing a broad role in learning and behavioral adaptation, although it need not be expected to be critical for the acquisition of all classes of learned habits.

An important goal for future research is to identify the nature of the cognitive deficit underlying the learning problems of rats with damage to structures comprising the General Learning System. For example, in a recent study, Thompson et al. (1989a) presented experimental evidence that deficits in certain forms of behavioral inhibition may have been crucial and that animals with damage to the General Learning System had difficulty in suppressing initial nonadaptive response patterns.

7. Age-Related Changes

Although most animal models of mental retardation deal with aberrant neurobehavioral development early in life, it is also true that significant problems can occur on the other end of the life span. Thus, aging in humans can result in severe and crippling neurobehavioral impairments, such as Alzheimer's disease and Parkinson's disease. It is becoming increasingly clear that also aged rats, like old people, can develop impairments with regard to cognitive behavior and motor functions. Although there is no evidence that aged rats suffer from true Alzheimer's disease or Parkinson's disease, the behavioral and neural changes are sufficiently similar to the human condition to warrant intensive study (*see* Gage and Björklund, 1986a).

As an example of the cognitive deficits seen in the aged rat, Geinisman et al. (1986) tested young adult (4 mo) and old (26 mo) male rats in an eight-arm radial maze (*see* Olton et al., 1979). The animals were first deprived of food to 80% of their normal body weight, and were then given one trial a day in the maze. Training continued until the subjects made no errors (i.e., no repeated entries into a particular arm within a trial) on three consecutive trials, or to a maximum of 30 trials. Whereas the young males mastered the problem after an average of 12 trials, 50% of the aged individuals failed to reach criterion within the 30 d of testing (Geinisman et al., 1986).

Another sensitive measure of impaired cognitive ability in the aged rat is provided by the water maze test devised by Morris (1984). Using this spatial learning task, in which rats learn to escape from opaque water onto a hidden platform, Gage et al. (1984b) tested 2-yr-old and young adult female rats. The performance of the aged subjects was markedly inferior to that of young rats: the aged rats swam a greater distance to reach the platform, and hence, their escape latencies were considerably protracted. It should be noted, though, that there was marked variation in the behavioral impairments seen between individuals of the aged rat population, severe deficits being seen in about one-third of the rats.

Gage et al. (1984b) also assessed the motor coordination skills of their aged subjects. Included in these tasks was the ability to maintain balance and reach a safety platform at either end of a narrow bridge. Young animals typically had little difficulty in walking along the rod, whereas most aged rats fell off the bridges or lay on the rod without attempting to walk (*see also* Gage et al., 1984a).

It has been found that the learning impairments seen in old rats in various learning tasks can be correlated to different types of brain pathology (e.g., Geinisman et al., 1986). For example, Gage et al. (1984b) reported that the learning impairments seen in aged rats were correlated to decreased glucose utilization in restricted areas of the limbic system. Thus, the decline in water maze performance correlated significantly with decrements in regional deoxyglucose utilization in the hippocampal formation, the prefrontal cortex, and the medial septum-diagonal band area. Because regional glucose consumption is partly dependent on the cholinergic innervation of these areas, it is interesting that major cholinergic cell groups in the basal forebrain (e.g., in the medial septum) undergo degenerative changes with age in the rat (Fischer et al. 1989). This deficiency in cholinergic projections, from the septal-diagonal band area to the hippocampal and prefrontal regions, may contribute to the reduced glucose utilization in these areas, as well as to the cognitive impairment in old rats (Fischer et al., 1989). Needless to say, it is of considerable interest in this regard that in humans afflicted by Alzheimer's disease, there is a marked loss of basal forebrain cholinergic perikarya (*see* Terry and Katzman, 1983) and that the severity of the dementia is correlated with the extent of the cholinergic deficiency (*see* Fischer et al., 1989).

Perhaps one of the most intriguing developments in the field of experimental aging research is the finding that the implantation of grafts prepared from fetal septal-diagonal band area gives rise to a new terminal cholinergic network into the aged host hippocampal formation (Gage et al. 1984a). In a detailed behavioral study on the learning performance of old rats bearing cholinergic septal grafts, Gage and Björklund (1986b) found that transplanted animals were significantly improved in the Morris water maze compared to their pretransplantation performance. Thus, the grafting procedure apparently restored the ability of old rats to use spatial cues to locate the hidden platform in the water tank. In a similar vein, it has been found that motor coordination skills of aged rats were improved after implantation in the striatum with dopamine-rich substantia nigra grafts (*see* Gage and Björklund, 1986a).

8. Differential Housing Environment During Development

Rats reared in impoverished environments demonstrate retarded development of several neurochemical and neuroanatomical markers compared with those animals reared in enriched environments (EC) (Bennett et al., 1964, 1970; Greenough, 1975; Krech et al., 1960; Rosenzweig et al., 1972). Bearing this in mind, it may be expected that several reports would demonstrate retarded learning performance by impoverished-reared (IC) rats and mice (Gardner et al., 1975; Hymovitch, 1952; Krech et al., 1962; Morgan, 1973) although not all appear to do so (Gill et al., 1966; Ough et al., 1972). EC-reared rats develop a heavier cerebral cortex, larger cell bodies, increased dendritic arborizations, a greater number of dendritic spines, and larger synapses (Greenough and Volkmar, 1973; Walsh et al., 1973). Neurochemically increased cholinesterase activity and acetylcholinesterase activity have been obtained (Rosenzweig and Bennett, 1977; Rosenzweig, 1984). The consensus seems to be that EC-reared rats are superior to those reared in IC in the performance of maze-learning tasks, and display lower levels of basal activity in open-field tests or in automated (photocell) test cages (Smith, 1972). Thus, it seems reasonable to assume that one model of mental retardation may be constituted by studying learning performance and brain correlates of IC relative to that of EC-reared rats.

9. Interactions of Differential Rearing Conditions with Neurochemical Lesions

O'Shea et al. (1983) and Pappas et al. (1984) first investigated the effects of NA depletion upon the differential effect of EC and IC on learning performance. They showed that rats injected with 6-OHDA (systemically 50 mg/kg just after birth to give an NA depletion) were less sensitive to the environmental rearing conditions than control rats, as measured by maze test running latencies. However, it should be noted that Whishaw et al. (1987) did not confirm those results. Subsequently, Saari et al. (1987) confirmed the previous findings (e.g., Pappas et al., 1984),

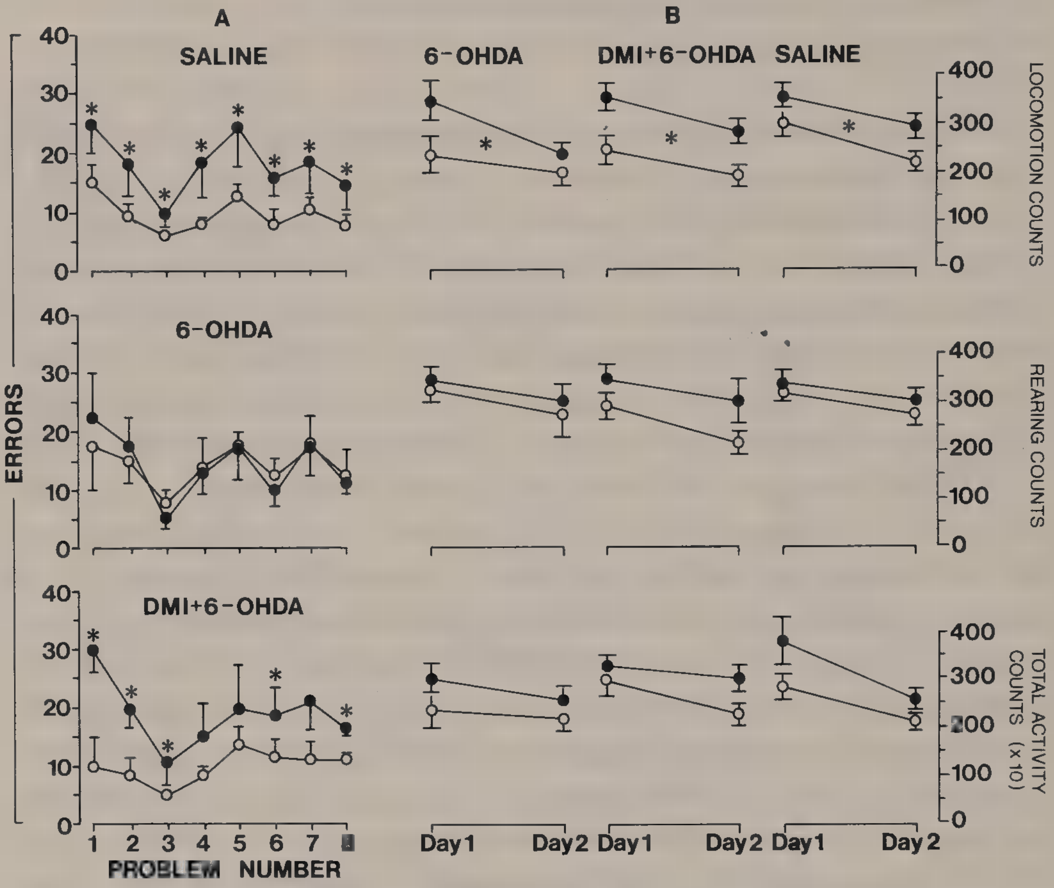

Fig. 6. Left-hand panel (A): Median (+ quartiles) errors per problem in the Hebb-Williams maze for 6-OHDA, DMI + 6-OHDA and saline-treated rats housed in an isolated (●—●) or an enriched (○—○) environment after weaning. Right-hand panel (B): Mean (+ SE) locomotion, rearing and total activity counts by the same groups tested in automated activity devices 1 wk after the maze test. (Redrawn from Mohammed et al., 1986a.)

indicating that neonatal NA depletion attenuated the improved spatial learning performance observed in the EC-reared rats.

Mohammed et al. (1986a) studied the effect of selective destruction of NA neurons by 6-OHDA (100 mg/kg, sc) on day 1 after birth upon the problem-solving performance of rats in the Hebb-Williams maze. As indicated below (*see* Fig. 6), the saline-treated control rats that had been reared in the EC made significantly fewer errors than those rats that had been reared in the IC. The pharmacological control condition DMI + 6-OHDA (desipramine, the NA uptake inhibitor, injected 30 min before 6-OHDA to protect NA neurons) produced a similar differential effect to the saline condition, i.e., EC better than IC. In the 6-OHDA condition, however, the performance of rats reared in the enriched environment was the same as that of those reared under IC. IC rats, independent of whether or not they were NA depleted, showed the usual hyperactivity associated with isolation or restricted housing.

The effects of 6-OHDA-induced neonatal DA lesions upon learning performance and motor activity of postweaning EC- and IC-housed rats have also been studied in several experiments (Mohammed et al. submitted). Neonatal DA depletions were inflicted through icv or ic injections of 6-OHDA (either 50, 75, or 200 μg) after pretreatment with desipramine to protect NA terminals. All the rats were tested as adults, i.e., between 70 and 100 d of age. DA-depleted rats showed marked hyperactivity and impairments in maze-learning performance; these deficits were significantly improved by postweaning housing in an enriched environment, but only as a function of the DA depletion. Severe DA depletions (>95% DA loss) did not lead to improvements following rearing in EC conditions. Figure 7 shows the effect of differential housing upon hyperactivity and water maze-learning impairments caused by neonatal 6-OHDA (50 μg) administration. Table 3 presents the monoamine concentrations of brain regions from rats treated neonatally with 6-OHDA (50 μg) on day 1 after birth.

10. Teratological Effects Related to Mental Retardation

10.1. Organic Tin Exposure

Teratological effects, which involve the incidence of abnormalities arising in prenatal development, usually involve rather major changes in the central nervous system (CNS) and in CNS function (Yanai, 1984). A huge variety of agents are known to induce behavior teratological effects, and many of the agents may be considered in animal models of retardation. Here, we will restrict the consideration to changes of spontaneous and learned behaviors brought about by the prenatal administration of the organic tins. In the rat, early postnatal administration of

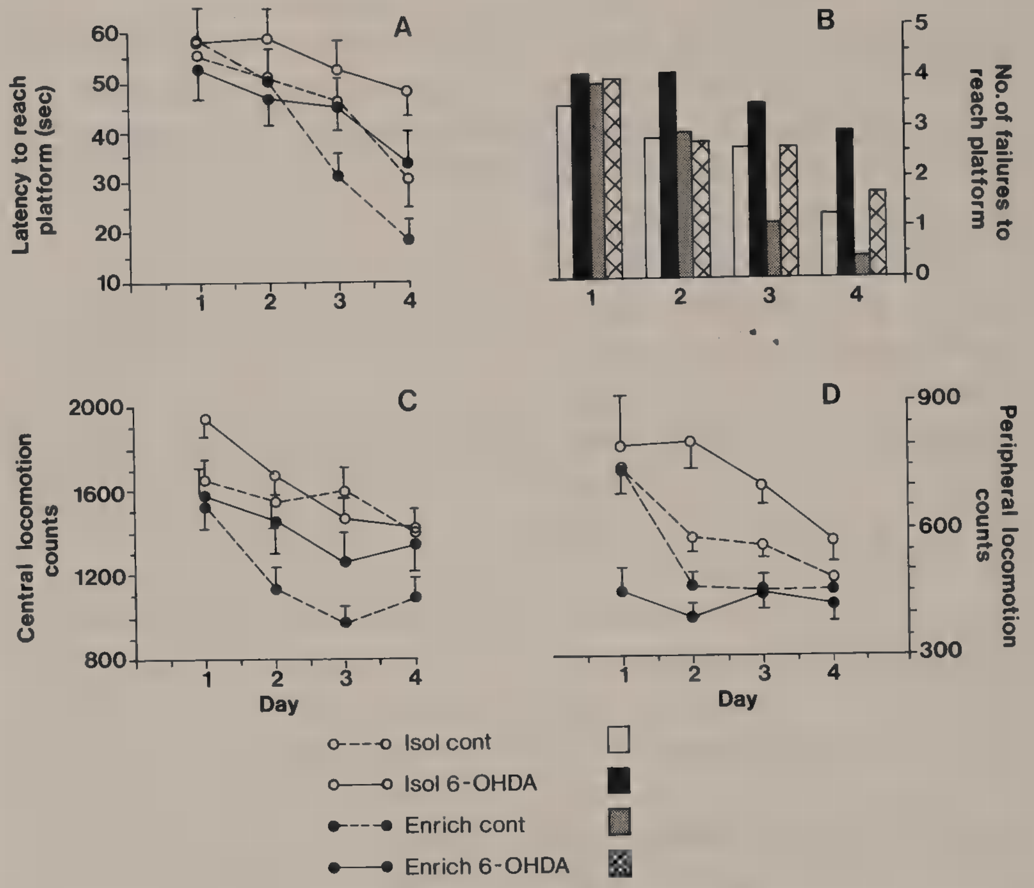

Fig. 7. Mean latency (+ SE) in the circular water maze to reach the platform (s) and number of failures to locate it (A and B); mean central and peripheral locomotion counts in automated open-field devices, 80 × 80 cm, (C and D) by neonatally treated 6-OHDA (ic 50 µg 30 min after DMI) and vehicle treated (ic vehicle 30 min after DMI) rats.

Table 3

Changes in Noradrenaline (NA) and Dopamine (DA) Concentrations (ng/g) in the Frontal Cortex, Striatum, and Accumbens of Adult Rats Treated Neonatally with 6-Hydroxydopamine (50 µg, ic) at Day 1

	Frontal cortex	Striatum	Accumbens	
	NA	DA	NA	DA
DMI + 6-OHDA[a]	288 ± 11	1776 ± 218**	587 ± 36	1134 ± 304**
(%)	(98)	(17)	(93)	(19)
DMI + Vehicle	293 ± 16	10216 ± 383	632 ± 62	6236 ± 693

[a]DMI = desipramine (25 mg/kg sc) injected 30 min before 6-OHDA.

**$p < 0.02$

triethyltin (TET), structurally related to tributyltin (TBT) and trihexyltin (THT), causes irreversible neuropathological changes (Bouldin et al., 1981; Veronesi and Bondy, 1986), resulting in permanent changes in cognitive and motor function (cf Walsh et al., 1982). We have studied the effects of prenatal administration of TBT (1 and 5 mg/kg) and THT (5 mg/kg) upon spontaneous motor activity and instrumental maze learning. In addition to the considerable hyperactivity shown in these offspring, the THT-treated offspring evidenced a clearly retarded acquisition of the radial arm maze task (Teiling-Gårlund et al., 1991; *see* Fig. 8). In conjunction with the biochemical results of this and other investigations (e.g., Alderidge and Street, 1970, 1971), it was concluded that the functional changes induced by the organic tins were suggestive of a relative hyperinnervation of dopaminergic pathways, perhaps related to alterations of cellular processes governing oxidative phosphorylation.

10.2. Alcohol Exposure

The discovery of developmental aberrations in children of alcoholic mothers initiated studies, both human and animal, concerning the effects of alcohol consumption during pregnancy on offspring development. During an initial stage, animal studies were aimed at examining the hypothesis that alcohol was the causative agent, since the clinical studies were confounded by other variables, such as socioeconomic status, undernutrition, and other drug abuse (cf. Rydberg et al., 1985). Since the demonstration that alcohol was the main cause for the induction of the Fetal Alcohol Syndrome (FAS), the efforts of animal research workers were directed at establishing an animal model of FAS. The diagnosis of FAS is based on the occurrence of the following main symptoms: growth deficiency, dysmorphic characteristics, and central nervous system dysfunctions (Clarren and Smith, 1978; Jones and Smith, 1973). In behavior, the CNS disturbances manifest themselves in mental retardation, delays in sensorimotor development, attentional deficits, memory disorders, hyperactivity, and sleep disturbances. The results of the animal studies on behavioral teratology of alcohol are in the main in

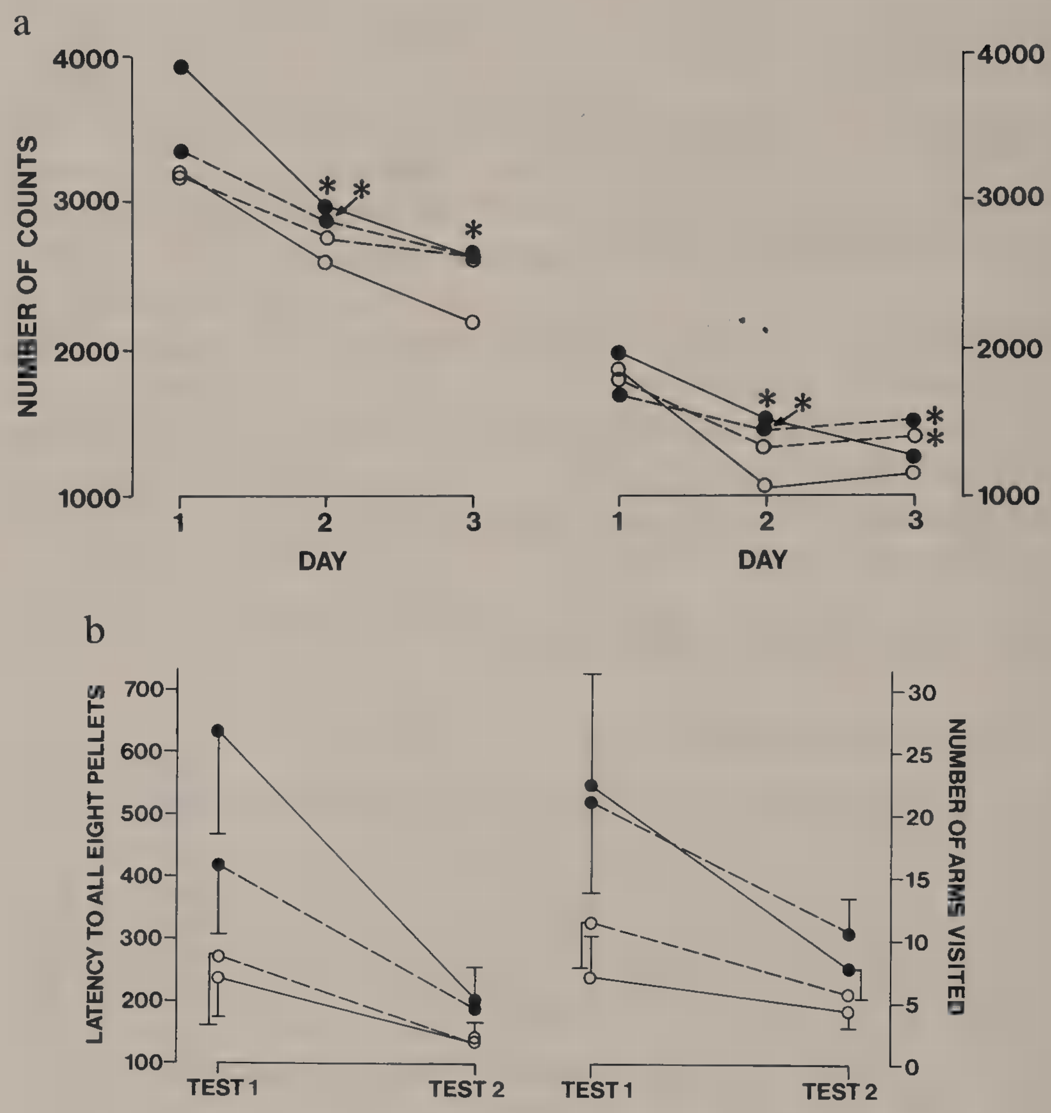

Fig. 8. (a) Spontaneous motor activity as indexed by total activity counts; (b) Latency to take all eight pellets and number of arms visited in a radial arm maze by rats treated prenatally with tributyltin and trihexyltin. Groups: control = ○—○, THT = ○- - -○, TBT(5) = ●- - -●, TBT (1) = ●—●.

accordance with those expected on the basis of clinical studies and constitute an animal model for experimental studies on FAS (Leonard, 1988; Meyer and Riley, 1986; Shah and West, 1984; West, 1986).

Several different procedures have been developed for the administration of alcohol to pregnant rodent mother. Alcohol is administered either during the whole pregnancy, or during a short, presumably critical period. Methods have also been developed for the administration of alcohol during neonatal and early postnatal ages (Samson and Diaz, 1982; Sonderegger et al., 1983). The reason for this is that a major portion of rat CNS development occurs postnatally. This applies especially to the development of the cerebellum and hippocampus, and to the brain growth spurt period. For this reason, the first postnatal week in the rat is approximately equivalent to the last trimester in the human. Besides the problem of procedures for the administration of alcohol, methodological issues to be addressed in each investigation are controls for undernutrition and/or stress, the necessity of foster-mothering after birth, sampling of animals for test, and adequate statistical treatments. Space limitation precludes a discussion of these issues, but excellent reviews discussing these aspects are available (Abel and Riley, 1986; Meyer and Riley, 1986).

Several studies have indicated that rat pups exposed to ethanol *in utero* display delayed sensorimotor development. Assessment of this development was performed by tests on negative geotaxis, surface-, and air-righting responses, ultrasonic vocalization, auditive function, and morphological landmarks like eye-opening and ear-flap unfurling (Church and Holloway, 1981; Hård et al., 1985; Kelly et al., 1987; Lee et al., 1980). For future studies, it is of special interest that rats exposed to high levels of alcohol on only 1 d of gestation, day 8, during the stage of gastrulation displayed disturbed development (Molina et al., 1987).

As noted above, hyperactivity is one of the cardinal symptoms of FAS and is also a rather common finding in animal studies on prenatal alcohol exposure. Overactivity has been observed in various test situations measuring motor activity in the open-field test (for reviews, *see* Bond, 1986; Fernandez et al., 1983; Meyer and Riley, 1986), and in running-wheel activity cages (Martin et al., 1978) or more specific exploratory activity like head-dipping or nose-poking (Riley et al., 1979). The manifestation of hyperactivity seems, however, to depend on the age of the fetal alcohol exposed (FAE) offspring at test. A summary statement of many studies indicates that FAE rats are hyperactive during preweaning and weaning ages compared to normal controls, show normal activity levels during young and adult age, and again exhibit hyperactivity in older age (Abel, 1982; Abel and Dintcheff, 1986; Bond, 1986).

During young ages when no spontaneous hyperactivity can be observed in the FAE rat, the basic trend towards hyperactivity may, however, be uncovered by treatment with amphetamine or methylphenidate (Blanchard et al., 1987a; Means et al., 1984). The drugs induced higher level of activity in FAE rats than in control animals, suggesting persistent dysfunction of the catecholamine systems caused by the prenatal alcohol exposure (*see above:* effects of prenatal MAM and organic tin treatments). Suggestive evidence for the involvement also of the cholinergic-inhibitory system in hyperactivity was provided by Bond (Bond, 1986). Finding that the cholinergic drugs scopolamine and physostigmine affected FAE and control rats differently, Bond concluded that prenatal alcohol exposure disturbed the development of the cholinergic system, thereby causing hyperactivity.

The observation of hyperactivity in FAE rats has been interpreted as a deficit in response inhibition caused by the prenatal alcohol exposure (Riley et al., 1986) If so, learning in test situations involving strong elements of response suppression should be especially difficult for FAE rats. This is also the case in studies using the passive avoidance conditioning situation (Abel, 1982; Driscoll et al., 1982; Gallo and Weinberg, 1982; Riley et al., 1979). FAE rats also show deficits in reversal learning in which the animal, after an initial period of training, has to learn to suppress the previously correct response in favor of a previously incorrect response (Anandam and Stern, 1980; Lee et al., 1980). Typically, this capacity is tested in a T-maze, where the animals first have to learn to go to one arm, e.g., to the left. During reversal training sessions the animal has to abandon the left-side turn, instead being rewarded for the right-side turn.

In learning situations where the purely associative elements are more directly assessed, FAE rats of early age exhibit difficulties in forming associations involving taste and odor stimuli (Barron et al., 1988; Riley et al., 1984). The typical experiment is to expose the animal for a taste or odor stimulus (conditioned stimulus), which is followed by an appetitive or aversive stimulus. A neonatal animal exposed to a distinctive smell, e.g., banana, in temporal contiguity with an oral infusion of milk, will later on show a specific preference for a location scented with banana smell. In the taste aversion paradigm, the consumption of a fluid flavored by a distinctive taste (e.g., saccharine) is paired with an injection of LiCl, causing nausea. By the association of saccharine with nausea, the animal later on display aversion for the saccharine solution. Difficulties in acquiring such associations are prevalent during neonatal and weanling stages in FAE rats compared to controls, but seem to be absent at adult age.

Only a few studies have utilized more complex learning situations, like labyrinths, to assess the possible occurrence of learning difficulties in prenatally ethanol exposed rats. Studies performed by help of Biel's water maze (Abel, 1979; Vorhees and Fernandez, 1986) and Hebb-Williams maze (Bond and DiGiusto, 1978) indicated no shortcomings in learning capacity of adult rats, prenatally exposed to ethanol. This may be because of the fact that, in the labyrinth situation, deficits in one psychological function may be compensated for by use of alternative strategies only involving functions that are intact. This may be a parallel to findings in human children of alcohol-consuming mothers, whose overall performance on global intelligence tests may be slightly affected in spite of easily observed difficulties in specific psychological functions, e.g., memory (Streissguth et al., 1989). This suggests that persistent effects of prenatal ethanol exposure are more easily detected by the help of tests assessing specific psychological functions than tests of a more global character (cf Olton, 1983).

Suggestive, but not conclusive, support for this notion is afforded by findings of Blanchard et al. (1987b). In previous studies, they observed similarities between FAE and hippocampectomized rats in learning difficulties, and changes in activity levels (Riley et al., 1986). They therefore suggested that prenatal alcohol exposure specifically affected the development of hippocampus. If so, prenatal alcohol exposure should also affect learning of the Morris water maze, specifically designed for assessment of spatial learning, which is strongly dependent on hippocampus. In accordance with their hypothesis, they found that the learning behavior of FAE rats in the Morris water maze was defective compared to normal controls (*see* Fig. 9). The experiment does not afford conclusive evidence for the generality of the prin-

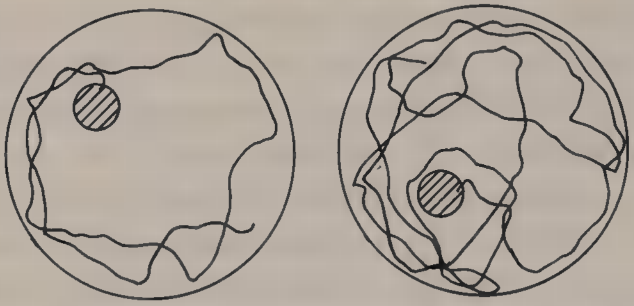

Fig. 9. Typical examples of swim patterns of controls (left) and prenatally ethanol-exposed rats (right) in the Morris water maze. Shown is the distance traveled to find the platform (strippled circle) hidden under the water surface. The figure depicts the behavior on day 2 of testing (trials 6–10). (Redrawn after Blanchard et al., 1987b.)

ciple stated above concerning assessment of specific vs global functions, since the experiment included only very young rats. It, therefore, cannot be excluded that the effects are age-dependent in the same way as for the detection of hyperactivity *(see above)*. In a recent study, Kelly et al. (1988) found impaired performance in the Morris maze in adult FAE females, but not in the males. Previously, they observed impairment in maze performance in both sexes at young age, suggesting an age-dependent effect also in this situation (Goodlett et al., 1987).

11. Conclusion

In the present chapter, a variety of potential animal models of mental retardation have been described, including prenatal nonselective neurotoxic or drug treatment, selective neurotoxic neonatal treatment, congenital models, brain lesions, differential housing, and age-related changes. Generally, the degree of reliability shown by these models assessing some extent of mental retardation is quite high, but the problem of the *validity* of the models is difficult to measure. It is only by circuitous comparisons of the IQ levels of certain known clinical syndromes (e.g., the FAS/FAE) in humans that the significance and/or extent of comparable learning and behavior deficits in animals may be indexed. Thus, for example, in humans, alcohol appears to produce a *mild* decrease in IQ level (*see* Fig. 10), and this may be compared with a mild to moderate behavioral deficit following

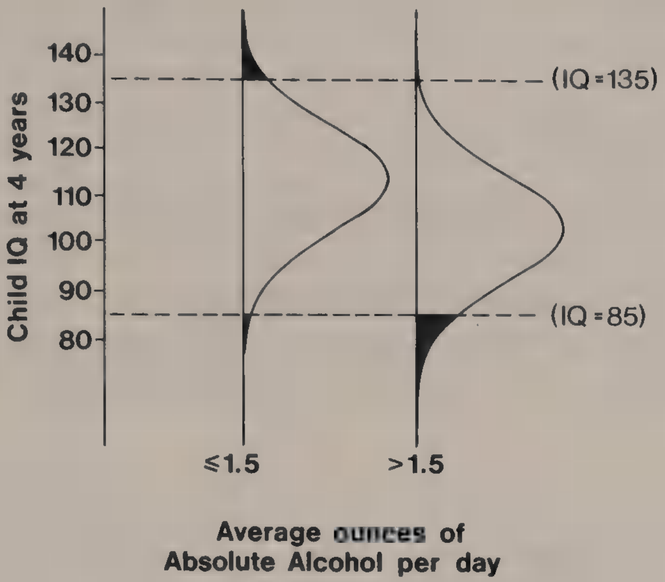

Fig. 10. Normal distributions demonstrating the effect of a mean decrease by five IQ points in children of mothers consuming more than 1.5 ounces of absolute alcohol per day (right part of the figure). The shaded areas depict the proportion of children outside the normal range of intelligence (IQ: 85–135). (Redrawn after Streissguth et al., 1989.)

the prenatal treatment of rats (e.g., Bond, 1986). Congenital models and neurotoxic treatments produce gross behavioral deficits and learning impairments, depending on the dose used and developmental period during which it is applied. It seems fair to deduce that these alterations are comparable to the moderate to severe scale of retardation as measured by IQ levels in human syndromes. It is uncertain whether rats with profound degrees of retardation are able to survive even in conditions of maximal maternal nursing. Clearly, during the postweaning period, rats with severe to profound retardation (possibly the icv 6-OHDA 2 × 100 μg preparation) require very careful extra feeding and nursing in order to survive. It is interesting to note that the enriched rearing/housing conditions do generally produce developmental improvements, although if the extent of damage (e.g., the drastic dopamine depletion produced by neonatal icv 6-OHDA, 2 × 100 μg) is too great no improvements as a result of environmental enrichment will be obtained. In conclusion, although it is clear that some promising models of mental retardation have been derived, the diversification of methods and treatments must indicate that the correct indices of validity should await further developments.

Acknowledgments

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Animal Models of Memory Disorders

David H. Overstreet and Roger W. Russell

1. Introduction

Disorders of learning and/or memory are commonly associated with Alzheimer's disease and are often regarded as among the earliest changes that can be detected in individuals developing this disease. However, several other disease states, such as Huntington's, Parkinson's, and Korsakoff's diseases, may also be associated with learning and/or memory disturbance. Changes in learning and memory may also be observed in individuals chronically exposed to such chemicals as ethanol and organophosphate pesticides, or following electroconvulsive treatment for depressive disorders. In fact, there is a wide array of conditions that may lead to a disruption of learning and memory in humans. Because of the complexity of typical human environments, it is not always possible to make clear-cut cause–effect statements, nor is it possible to indicate which brain regions, if any, are responsible for the behavioral deficits. For this reason, animal models of the various human conditions have been developed. In the present chapter, we will survey the approaches that have been used to disrupt memory processes in animals, the tasks used to assess memory processes, and the procedures developed to attempt to overcome the memory disturbance in animal models. We will cover more than just animal models of Alzheimer's disease, but do not claim to be all-inclusive.

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2. The Nature of Memory

2.1. Brief History

Interest in human memory processes has a long history, dating back to speculations of the Greeks and Romans. However, the systematic study of memory processes and memory disorders did not begin until the nineteenth century. This early work culminated with the consolidation hypothesis of Mueller and Pilzecker (1900) at the turn of the century. This model, which proposes that time is required before memories can be laid down in some form of permanent storage, has directed much of the neurobiological research into memory function in both animals and humans. One example of the type of study that has been conducted is the extensive work on the effects of electroconvulsive shock (*see* McGaugh and Herz, 1972); this work will be summarized in Section 3.3.

One of the outcomes of the consolidation hypothesis of Mueller and Pilzecker was the notion of at least two forms of memory: short-term and long-term. Hebb (1949) proposed that there were different neurobiological substrates underlying the two forms: a reverberating circuit was responsible for short-term memory, and structural changes in the nervous system were responsible for long-term memory. In more recent years, Gibbs and Ng (1977) have proposed more specific biochemical mechanisms underlying a three-stage model of memory (*see* next section). All of these studies have generally supported the view implicit in the consolidation hypothesis: that memory processes are heterogeneous and that these heterogeneous processes are mediated by different neurobiological mechanisms.

2.2. Theoretical Models

There have been a tremendous number of theoretical models developed to account for learning and memory processes; it is not our intention here to provide detailed descriptions of each of these models, but simply to point out that such models have been useful not only in accounting for the results of many prior experiments, but also in directing further experiments. Kesner (1986) has cogently summarized a number of neurobiological models of memory, including those of Karl Lashley, Richard Thompson, Mortimer Mishkin, Larry Squire, and his own. Squire (1987) has also summarized many of the neurobiological views of memory, including his own. Essential to the majority of these models are the notion that multiple brain systems participate in the encoding of memory and the recognition that there are multiple forms of memory.

One example is the distinction between short-term and long-term memory, which may be called by different names in the different models. Olton (1983) and others have used the terms working (short-term) and reference (long-term) memory with generally good results. Still other workers have suggested the existence of more than two memory traces (e.g., Gibbs and Ng, 1977; McGaugh, 1966). Gibbs and Ng, working with an inhibitory conditioning model in young chicks, proposed a three-trace memory model in which immediate memory was associated with potassium ion fluxes, short-term or intermediate memory was associated with the sodium-potassium pump, and long-term memory was associated with protein synthesis. Other investigators had previously suggested an association between protein synthesis and long-term memory; the new aspect of the Gibbs/Ng model was the postulation of specific biochemical correlates of immediate and short-term memory.

Thus, there have been a considerable number of theoretical models that have attempted to account for memory processes in terms of neuroanatomical and neurochemical correlates. In addition, there have been a number of models that have attempted to relate the behavioral concepts of learning and memory to other behaviors. One information processing model proposes that acquisition (learning) of information is dependent on other processes, such as arousal and attention, and that retrieval (memory) is dependent on personal biases and level of arousal (*see* Thompson, 1975). When attempting to develop an animal model of memory disturbance, it is important to keep in mind that manipulations that alter arousal and/or attention may have secondary influences on memory processes.

Finally, many theoretical models of memory processes recognize that they are heterogeneous. The neuroanatomical and neurochemical correlates of passive avoidance memory, for example, may be different from those underlying working memory in a radial arm maze. One consequence of this recognition is the realization that multiple tasks, both those reflecting memory processes and those reflecting other behavioral processes, must be used to validate suspected animal models of memory disorders.

2.3. Human Memory Disorders

Although the most common disease associated with a disorder of memory in humans may be Alzheimer's disease, it is important to realize that quite a number of other conditions may result in memory disorders as well. Among the conditions that have been associated with an impairment of memory are neurological disorders, such as Huntington's and Parkinson's diseases (Sahakian et al., 1988), global amnesias associated with surgical removal of brain regions, accidents, or alcohol abuse (e.g., Korsakoff's disease), transient memory loss associated with electroconvulsive therapy (ECT) for neuropsychiatric disorders, and confusion and memory disturbance associated with chronic drug exposure, e.g., organophosphates. Interestingly, both severe depression and ECT may produce an apparent memory disturbance, but these can be distinguished by careful testing (McAllister, 1981). Similarly, the "pseudodementia" associated with depressive disorders can be distinguished from the dementia associated with Alzheimer's disease by appropriate testing. A number of investigators have attempted to compare the degree of memory impairment associated with the different disorders and have frequently found that a superficial similarity disappeared upon further, more detailed testing (e.g., Beatty et al., 1988; Corkin, 1982; Cranholm and Butters, 1988). Such findings reinforce the point made above that multiple testing procedures should be carried out in order to arrive at any conclusions.

The mechanisms by which these various conditions produce memory disorder in humans have not been conclusively established in many instances, hence, the need for animal models. Rather than summarize the literature concerning humans that has led to speculations about how the various conditions mentioned above produce memory disturbance at this point, we will bring in the relevant literature when the appropriate animal models are discussed. Although it is widely recognized that memory problems are associated with aging in both humans and animals, space does not permit us to summarize this voluminous literature here. The interested reader may wish to refer to any number of recent reviews, particularly the recent special issue of *Neurobiology of Aging* (e.g., Bartus, 1988; Mohs, 1988).

2.4. Animal Models

Animal models have provided much valuable information about human conditions. Developing models for the wide variety of conditions that result in memory disorders has proved extremely challenging for a number of reasons. In many cases, the etiology of the disease resulting in the memory disorder is unknown. Second, as indicated above, memory processes are heterogeneous and are closely related to other behavioral processes, so it has been difficult to develop treatments that have a specific effect on memory processes. Third, the complexity of memory processes may be greater in humans, making it difficult to simulate human learning conditions in infrahuman animals. Nevertheless, certain human conditions are quite well understood, and suitable animal models have contributed to their understanding. In fact, a considerable number of review papers on animal models of memory disorders have appeared in the past few years, and our chapter has profited from the information presented in these reviews (Bartus et al., 1982,1986; Coyle et al., 1983,1984; Fisher and Hanin, 1986; Olton and Wenk, 1987; Overstreet and Russell, 1984; Pepeu and Pepeu, 1988; Smith, 1988; Walsh and Chrobak 1990; Wenk and Olton, 1987). In the sections below, we will describe the approaches that have been used, their limitations, and their contributions to our understanding of memory disorders.

3. Techniques for Impairing Memory

Learning and memory are behavioral concepts that are inferred from the performance of animals and humans. Consequently, they can be regarded as related to other basic properties of living organisms: neuroanatomical, electrophysiological, and biochemical (e.g., Russell, 1966). It is not surprising, therefore, that each of the above approaches has been used in an attempt to disrupt memory in animals. In fact, the literature is so vast that it is impossible to cover all of it in this short chapter. Rather, those approaches that have been designed to model a specific human memory problem will be singled out. Other relevant material may be covered in somewhat less detail.

3.1. Surgical Lesions

The surgical removal of brain areas or cutting of fiber tracts is the time-honored way to study the behavioral consequences of brain damage. This approach was responsible for many of our early ideas about brain function and behavior (e.g., Lashley, 1950). Olton (1986) has summarized the theoretical basis and utility of the lesion approach to study memory processes, and the reader is referred to his chapter for additional information. In this section, the use of surgical lesions to develop animal models of memory disorders will be examined.

One of the debates in the field of human memory disorders relates to the anatomical regions responsible for the memory disturbance seen in global amnesiacs. It was well known that both Korsakoff's patients, with presumed damage in the diencephalic areas, and global amnesics, with presumed temporal lobe damage, had similar behavioral deficits on many behavioral tasks. More recent studies, using more sophisticated behavioral approaches, have revealed some differences between these two subgroups, but the notion of two groups of global amnesics has been retained (*see* Squire, 1987).

Attempts to develop animal models of these two subtypes of global amnesia overwhelmingly favor the temporal lobe condition. Even so, there was a protracted debate about the relative roles of the amygdala, the temporal stem, and the hippocampus in producing memory disturbance, since Mishkin (1978) demonstrated much more severe memory disturbance in monkeys following the combined lesion of the amygdala and the hippocampus. Since the temporal stem was commonly damaged upon removal of the amygdala, it was argued by some that this area was the critical one for the observed behavioral deficits (*see* Overstreet and Russell, 1984, for references). However, more recent techniques that have selectively damaged the amygdala or the temporal stem have concluded that the amygdala and the hippocampus were the critical regions and conjoint damage produced a greater behavioral deficit (Squire, 1987).

The majority of the above studies used primates as the experimental animal and suction (aspiration) as the means of lesion. Other surgical lesion approaches in common use are knife cuts of specific pathways, and electrolytic or radio-frequency lesions of particular regions. Although these approaches have led to a considerable amount of data on anatomical regions underlying particular behavioral functions, there are inherent and continual problems with interpretation of findings. The most common criticism of lesion-based approaches is the lack of specificity. All approaches, except possibly the knife cuts, damage both cell bodies in the area and axons passing through the area. It is difficult, therefore, to determine the relative contributions of the cell bodies and the axons of passage to the behavioral deficit. Because of these problems with interpretation and because of the development of neurotoxins with varying degrees of selectivity, no attempt has been made to summarize the various parameters used in the surgical lesion approaches, which have been largely supplanted by approaches using neurotoxins (but *see* Markowska et al., 1989).

3.2. Neurotoxic Lesions

There is now a wide range of neurotoxic agents available: excitotoxic amino acids, such as ibotenic acid, catecholamine toxins, such as 6-hydroxydopamine, indoleamine toxins, such as 5,7-dihydroxytryptamine, cholinergic toxins, such as AF64A, and tubulin-binding toxins, such as colchicine. There is also a considerable amount of literature on each of these toxins; Schallert and Wilcox (1985) reviewed some of the previous literature in an earlier volume of this series. In the present section, the focus will be on three groups of chemicals that have been most commonly used to induce some form of memory deficit: the excitotoxins; the cholinotoxin, AF64A; and colchicine.

3.2.1. Excitotoxins

This group of compounds is related to the naturally occurring excitatory amino acid, glutamate, and is thought to have selective toxic effects on the cell bodies in a brain region. Kainic acid was the first of this group to be used widely, but it is generally regarded to have a very steep dose–response curve and, consequently, often results in nonspecific damage; it may also produce damage in the CA1 and CA3 areas of the hippocampus when injected elsewhere (Wuerthele et al., 1978). Therefore, it is not a particularly useful tool for producing animal models of memory disturbance (*see* Ermakova et al., 1989). More promising results have been obtained with quinolinic acid, an endogenous excitotoxin that has been implicated in Alzheimer's disease (Schwarcz and Meldrum, 1985), and ibotenic acid, a naturally occurring excitatory amino acid that has greater selectivity than kainic acid (Kohler and Schwarcz, 1983).

By far, the large majority of papers have utilized ibotenic acid and have selected the nucleus basalis magnocellularis (NBM) as the region to lesion because of the evidence implicating this region in Alzheimer's disease (*see* Coyle et al., 1983). The drug is administered in anesthetized rats using a stereotaxic instrument and a 30-gage injection needle. The typical volumes are 0.5–1.0 μL of a solution of about 0.06*M*. To assess the effect of this treatment, it is common to measure the activity of the synthesizing enzyme, choline acetyltransferase (CAT), in the cerebral cortex. Some investigators have also studied sodium-dependent, high-affinity choline uptake (HACU) and the activity of the metabolizing enzyme, acetylcholinesterase (AChE). Often the decrease in CAT activity is greater than 40%, suggestive of a dramatic loss of cholinergic neurons projecting to the cortex.

According to summaries of the older literature by Coyle et al. (1984) and Smith (1988), lesion of the NBM with ibotenic acid reproduces many of the deficits seen in Alzheimer's disease, including significant reductions in CAT activity, HACU, and AChE activity as well as cognitive deficits. Some workers have compared the effects of NBM lesions with those of the medial septum or fimbria-fornix, which contains the cholinergic axons from the medial septum to the hippocampus (e.g., Dunnett, 1985;

Dunnett et al., 1989; Etherington et al., 1987; Hepler et al., 1985a; Meck et al., 1987). These studies have suggested the involvement of NBM-cortical pathways in long-term or reference memory, and the septohippocampal pathways in short-term or working memory. However, there is no consensus on this point, and more recent studies have even called into question the involvement of cholinergic systems in NBM-lesioned animals (e.g., Dunnett et al., 1987; Evenden et al., 1989).

One of the problems with the excitotoxins is that, although they tend to be axon-sparing, they have nonspecific effects on cell bodies. Thus, even though the NBM may contain a high proportion of cholinergic neurons, cell bodies containing other neurotransmitters may also be damaged. Several recent studies have reported that quisqualic acid injections into the NBM produced comparable deficits in cortical cholinergic markers, but less nonspecific damage than ibotenic acid (Dunnett et al., 1989; Everitt et al., 1987; Robbins et al., 1989; Wenk et al., 1988). More importantly, these studies also reported that the behavioral effects of quisqualic-lesioned rats were reduced, suggesting that the behavioral deficits associated with ibotenic acid lesions were the result of damage of noncholinergic neurons (e.g., Dunnett et al., 1989). Wenk et al. (1988) have recently implicated neurotensin as one potential candidate of the noncholinergic neurotransmitter mediating these effects.

In sum, the very nice animal model of Alzheimer's disease represented by NBM lesions, as suggested in reviews by Smith (1988) and Olton and Wenk (1987), has been called into question by more recent studies (*see* Kesner, 1988). These reports have questioned whether the behavioral deficits induced by the lesions were truly the consequence of disruption of cholinergic functions or whether they might have been mediated by some other noncholinergic neurotransmitter, such as neurotensin (Dunnett et al., 1989; Robbins et al., 1989; Wenk et al., 1988).

3.2.2. AF64A

Ethylcholine mustard aziridinium ion (AF64A) is a structural analog of choline and is believed to produce toxic effects on cholinergic neurons by interaction with the choline uptake site (Chrobak et al., 1989; Mantione, 1981, 1984). A continuing debate exists regarding the specificity of this compound for cholinergic neurons. A significant number of investigators who have injected the drug directly into particular brain regions have reported varying degrees of nonspecific damage (e.g., Allen et al., 1988; Bailey et al., 1986; Kozlowski and Arbogast, 1986; Levy et al., 1984; McGurk et al., 1987; Stephens et al., 1987), but there is a disagreement whether it is possible to select a dose of AF64A that will be selective for cholinergic neurons (cf Kozlowski and Arbogast, 1986; McGurk et al., 1987). On the other hand, investigators who have used the intraventricular (2 or 3 nmol/side) route of administration of AF64A have commonly reported relatively specific effects on hippocampal cholinergic neurons (Chrobak et al., 1986,1988; Eva et al., 1987; Fisher and Hanin, 1986; Gaal et al., 1986; Hortnagl et al., 1987a,b; Jarrard et al., 1984; Tagari et al., 1986). Not only is there a lack of observable effects on noradrenergic neurons, but also it appears that depletion of noradrenaline can protect cholinergic neurons in the hippocampus from destruction by AF64A (Hortnagl et al., 1989).

Thus, although there will remain detractors, AF64A appears to produce relatively selective cholinergic deficits in the hippocampus, especially when administered intraventricularly. Interestingly, the deficits appear to be primarily for working memory (Chrobak et al., 1986,1988)—a result that is consistent with the lesion data discussed in the previous section. There do not appear to be any studies that have looked at the behavioral effects of direct injection of AF64A into cholinergic cell bodies (NBM and medial septum), even though there is evidence for relatively specific cholinergic effects (Kozlowski and Arbogast, 1986). In a recent preliminary study, we have compared the effects of intraseptal AF64A against intrahippocampal AF64A and septal electrolytic lesions on radial arm maze performance. The intraseptal AF64A administration produced a more dramatic impairment of working memory than did the other treatments (Bailey and Overstreet, 1988). Further work in this area is required, because at present, the only selective mode of administration of AF64A, the intraventricular route, results in only a loss of hippocampal cholinergic neurons. The large loss of cortical cholinergic neurons seen in Alzheimer's disease (Coyle et al., 1983) has not been mimicked by AF64A treatment as yet (*see* Tagari et al., 1986).

Even though there remains some argument about the specificity of the biochemical effects of AF64A, there is no doubt about its effect on cognitive behavior. There are deficits in both acquisition and retention of a passive avoidance task (Bailey et al., 1986; Walsh et al., 1984), performance in the Morris water maze (Brandeis et al., 1986), and as indicated above, working memory in the radial arm and multiple component T mazes (Chrobak et al., 1986,1988). There can also be short-term changes in other behaviors, such as motor hyperactivity (Bailey et al., 1986; Jarrard et al., 1984; Walsh et al., 1984). Recently, we have explored the time-dependent changes in cholinergic sensitivity in hippocampally infused AF64A-treated rats (Schiller and Overstreet, 1987). We noted an increased sensitivity to the agonist oxotremorine and a reduced sensitivity to the antagonist scopolamine several weeks after treatment—data consistent with cholinergic supersensitivity, as expected in lesioned rats. However, at about 10 wk after AF64A treatment, the AF64A-treated rats were subsensitive to oxotremorine and supersensitive to scopolamine (cf Sunderland et al., 1988). Thus, there are time-dependent adaptive changes that must be taken into account when interpreting the effects of lesions. It is possible that these adaptive changes contribute to behavioral recovery.

3.2.3. Colchicine

The demonstration that colchicine, which binds to tubulin, has selective neurotoxic effects occurred 10 yr ago (Goldschmidt and Steward, 1980), but the behavioral consequences of this treatment have only recently been studied. The greater susceptibility of granule cells in the dentate gyrus to damage by colchicine has now been widely replicated and documented (Dashieff and Ramirez, 1983; Goldschmidt and Steward, 1982; Steward et al., 1984; Tilson and Peterson, 1987; Walsh et al., 1986). In fact, although there is an extensive loss of granule cells following the administration of 3.5 μg of colchicine into the dentate gyrus, the pyramidal cells and the γ-aminobutyric acid (GABA) interneurons are virtually intact (Mundy and Tilson, 1989; Walsh et al., 1986). A number of studies indicate that colchicine injections into other brain regions may also result in neuronal damage, but the effects are generally not as selective as those in the hippocampus (Goldschmidt and Steward, 1982; Mundy and Tilson, 1989). So there is a potential for the use of colchicine in other brain areas to damage selectively certain cell populations.

The intradendate injection of colchicine has led to several behavioral changes in rats that are consistent with what is known about hippocampal function. Thus, there are increases in motor activity and startle reactivity as well as deficits on various learning and memory tasks, such as passive and active avoidance and the radial arm maze (McLamb et al., 1988a,b; Mundy and Tilson, 1989; Tilson et al., 1987, 1988a,b; Walsh et al., 1986). However, as consistent as these results are, there is still a problem in the model because the granule cells are not the primary cell population lost in Alzheimer's disease. Consequently, certain investigators, encouraged by preliminary reports on the vulnerability of cholinergic neurons to colchicine (Peterson and McGinty, 1988), are beginning to study the effects of colchicine administered directly into regions of the brain rich in cholinergic cell bodies.

Mundy et al. (1989) reported that colchicine (1 μg/site, bilateral) injection into the NBM resulted in a 29% decrease in cortical CAT activity, a loss of NBM cholinergic neurons without any apparent other damage, a large deficit in passive avoidance memory, and a transient deficit in the water maze—findings very similar to those reported above after excitotoxic lesions of the NBM. Mundy and Tilson (1988) compared separate hippocampal and NBM lesions with combined lesions, and suggested that combined lesions more closely reproduce the cognitive deficits seen in Alzheimer's disease. In a third study, Tilson et al. (1988a) reported that colchicine dose-dependently produced radial arm maze deficits following NBM injections, but that the treatment also reduced cortical levels of noradrenaline and other biogenic amines. From this perspective, the treatment more closely mimics Alzheimer's disease than does treatment with the excitotoxins. Thus, the initial studies are promising, but more work is needed.

3.2.4. Concluding Remarks

Although it is clear that excitotoxins, AF64A, and colchicine all produce neurodegenerative and cognitive effects, with varying degrees of specificity, it is still unclear which approach would be the best if one were attempting to develop an animal model of Alzheimer's disease. The model produced by excitotoxin administration into the NBM suffers from two problems in addition to those mentioned above: the transient nature of the cognitive deficits and the relative ease of improvement with cholinergic agents (*see* Smith, 1988; Section 5.1.). As mentioned above, little attention has been given to the injection of AF64A into brain regions rich in cholinergic neurons; therefore, present findings with this compound may only be relevant to hippocampal cholinergic deficits. Finally, although recent studies indicate that NBM lesions can be induced by the central injection of colchicine (e.g., Tilson et al., 1988a), it is still too early to determine whether colchicine has any significant advantages over the excitotoxins ibotenate and quisqualate. Nevertheless, these approaches are certainly in the right direction and, as we will see in Section 5, have been extensively used in attempts to detect memory-enhancing effects of particular treatments.

3.3. Electrical Stimulation

It is well known that electrical stimulation, both globally to produce seizures and locally to specific brain regions, may result in memory deficits (McGaugh and Herz, 1972; McGaugh and Gold, 1976). In fact, memory complaints from individuals undergoing electroconvulsive therapy (ECT) are quite common (Squire, 1987), and studies of the memory deficits of such individuals have revealed a great deal about the postulated role of temporal lobe function in memory processes (Squire, 1987). One of the difficulties with the study of this area in humans is that those individuals who are being treated by ECT are often treatment-resistant depressive patients who may suffer from a type of cognitive deficit referred to as "pseudodementia." However, McAllister (1981) has reported that special behavioral tests can be used to differentiate memory loss resulting from ECT from the cognitive changes associated with depression. More recently, Squire and Zouzouni (1988) have developed a self-rating questionnaire that differentiates depressives from global amnesics. It is therefore quite clear that ECT produces a memory deficit in humans that can be differentiated from the cognitive changes associated with depression.

Because regions of the temporal lobe, such as the amygdala, the hippocampus, and the dentate gyrus, are very susceptible to seizure, these areas have been implicated in the memory-disruptive effects of ECT (Squire, 1987). It has also been established that electrical stimulation of the specific regions can also have memory-disruptive effects (e.g., Collier et al., 1982; Zornetzer et al., 1974). In fact, the memory deficits seen in patients after ECT treatment, although transient, bear a striking resemblance to those seen in global amnesics with the bitemporal syndrome (Squire, 1987). Therefore, it may be that both the amygdala and the hippocampus participate in the cognitive dysfunctions seen in ECT-treated individuals. There do not appear to have been any animal studies that have attempted to address this issue of which brain region may be critically involved in ECT's disruptive effects on memory.

Other investigators have studied the effects of electroconvulsive shock (ECS) on animals in an attempt to understand its antidepressant effects. Relatively few of these studies have commented on the memory-disruptive effects of ECS, although their findings are clearly relevant to the mechanisms underlying ECS and ECT. A wide range of neurotransmitter-related changes have been observed, including decreases in the concentration of acetylcholine muscarinic receptors (mAChR), β-adrenergic receptors, and serotonin S2 receptors (Gleiter and Nutt, 1989). Lerer (1985) has speculated that the decrease in mAChR is the most likely mechanism underlying the memory disruptive effect of ECS. This speculation is based on a reasonable amount of literature correlating changes in mAChR concentrations with memory function: treatments that increase mAChR density may lead to an enhancement of memory (Loullis et al., 1983), whereas those that decrease mAChR density may lead to a disruption (Gardner et al., 1984; Overstreet et al., 1988). This loss of receptors may account for the lack of success in using cholinergic agents, like physostigmine, to reverse the memory deficits in ECT-treated patients (Squire, personal communication).

In general, there has been less interest in developing an animal model of ECT-related memory disturbance, and most studies of the changes occurring in ECS-treated animals have been more interested in the mechanisms underlying the antidepressant effects (Gleiter and Nutt, 1989). Therefore, no statement more conclusive than the postulation of decreases in mAChR density mentioned above can be made at this time. More research is necessary, particularly because unilateral ECT, which does not produce a memory deficit, does not seem to be as effective an antidepressant treatment as was formerly thought (Nemeroff, personal communication).

3.4. Dietary Restriction

Dietary factors have occasionally been implicated in the etiology of memory or other disorders. Examples include the association of aluminum with Alzheimer's disease, cycads with motor neuron disease, and lack of thiamine with Korsakoff's disease (Mair et al., 1988a; Pettegrew, 1986; Spencer 1987). Space does not permit a full discussion of all of the possible dietary models. The two models that will be expanded upon in this section are the thiamine-deficiency model and the acetylcholine-deficiency model.

3.4.1. Thiamine-Deficiency Model

This model was considered in some detail in an earlier review, where it was pointed out that there was a dispute whether cholinergic or serotonergic mechanisms were responsible for the cognitive deficits (Overstreet and Russell, 1984). Only the more recent papers will be considered in this section, particularly those of Mair and associates (e.g., Mair et al., 1988b).

Thiamine deficiency may be induced in experimental animals by feeding them a test diet of thiamine-deficient food and simultaneously injecting them with the enzyme inhibitor pyrithiamine (0.5 mg/kg in rats) to prevent the endogenous synthesis of thiamine (Mair et al., 1988a). Animals may be studied in the deficient state, which normally occurs between 12 and 15 d of treatment, or at some time later after a normal diet has been resumed. More commonly, studies of the recovered animals are conducted, because the consequences of acute thiamine deficiency are so extreme and nonspecific.

Animals that have recovered from a period of thiamine deficiency are known to have permanent brain lesions (e.g., Irle and Markowitsch, 1982; Witt and Goldman-Rakic, 1983a), long-lasting changes in brain neurotransmitter levels (e.g., Langlais et al., 1987,1988), and impairment on the acquisition of an object nonmatch to sample task (Witt and Goldman-Rakic, 1983b) or in spatial delayed alternation (Irle and Markowitsch, 1982; Mair et al., 1985). However, until the report of Mair et al. (1988a), no investigators looked at all three variables in the same group of animals. Mair et al. (1988a) confirmed the presence of a high incidence of medial thalamic lesions in the rats recovered from thiamine deficiency and a selective depletion of noradrenaline in some brain regions. However, they determined that the behavioral deficits were selective, being seen only on spatial nonmatching to sample tasks and not upon simple discrimination tasks (Mair et al., 1988a). This pattern of deficits is comparable to those seen in humans with Korsakoff's disease (Oscar-Berman and Zola-Morgan, 1980; Oscar-Berman et al., 1982). So, too, is the impaired olfactory learning (Mair et al., 1988b). Thus, the recovered thiamine-deficient rat may be a useful model for Korsakoff's disease because it simulates the neuropathological and behavioral changes.

The noradrenergic deficits seen in the recovered rats would also be consistent with the evidence for noradrenergic deficits in Korsakoff's patients (e.g., McEntee and Mair, 1978, 1980; Mair and McEntee, 1983). As yet, there do not appear to have been any attempts to counteract the memory impairment in the rats with noradrenergic agonists, as there have been in humans. Certainly such studies are important in establishing the utility of the model. Also, Langlais et al. (1988) have recently reported changes in the regional concentrations of other putative neurotransmitters, such as glutamate and GABA, so it may be premature to ascribe the cognitive deficits observed in thiamine deficiency solely to a noradrenergic deficiency. This promising model of Korsakoff's disease is certainly worth more attention.

3.4.2. Acetylcholine-Deficiency Model

Our earlier discussion of the cholinergic neurotransmitter system in memory has focused primarily on animal models involving morphological (structural) insult to the nervous system. Structures provide sites in which dynamic biochemical and electrophysiological events occur. The storage and retrieval of memories may be significantly disorganized when these normal dynamic events malfunction. The animal model (Jenden et al., 1987) discussed in the following paragraphs provides an experimental paradigm in which a false precursor, *N*-aminodeanol (NADe), is substituted for the natural precursor, choline (Ch), in the animal's diet. This leads to the synthesis of a false transmitter, *O*-acetyl-*N*-aminodeanol (AcNADe), and to significant deficiencies of memory.

The possibility that a false precursor leading to the synthesis of a false transmitter could serve as a pharmacological "tool" for studying molecular events in neurotransmitter systems began to receive attention some half-century ago. Later, it was appreciated that such tools might also provide animal models for studying clinical states involving dysfunctions in the brain. A few experiments using such models were designed to measure effects on behavior, both normal and abnormal. These results have been the subject of several reviews (Collier et al., 1979; Newton and Jenden, 1986; Whittaker and Luqmani, 1980).

During the past 5 yr, one compound has emerged that satisfies fully the requirements of a false precursor (Newton and Jenden, 1986): the precursor, *N*-amino-*N*,*N*-dimethylaminoethanol (NADe), is taken up by the Ch transport system in competition with Ch. It is acetylated by the enzyme, CAT, stored as AcNADe in vesicles, and released upon stimulation. Acetylcholine (ACh) stores are depleted as they are replaced by AcNADe. When released from presynaptic vesicles, AcNADe binds to both muscarinic and nicotinic receptors, and is hydrolyzed by AChE. AcNADe's potency at these receptors is only 4 and 17% of ACh, respectively. The effect is a profound interference with cholinergic transmission, thus producing a hypocholinergic state.

Most of the research on effects of NADe has used the laboratory rat as the animal model, although some preliminary studies have been conducted with mice. The method for preparing NADe has been described by Newton et al. (1983). Its structure and purity were confirmed by elemental analysis, nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GCMS). When substituted for Ch in the diet of rats immediately after weaning, NADe has been shown to be incorporated into phospholipids in plasma, liver and brain. After 60–120 d, free Ch is 85–95% replaced by free NADe, and lipid-bound Ch by 65–75%. CAT activity is reduced in the striatum, hippocampus, and cortex as well as in peripheral tissues, e.g., the ileum.

These characteristics of a hypofunctioning cholinergic system are reflected in a variety of behavioral and physiological functions known to involve that system (*see* Russell, 1982). Among these are the processes underlying memory. Early pilot studies in the UCLA laboratory indicated that long-term substitution of NADe for dietary Ch was reflected in concomitant changes in behavior, apparently including cognitive functions (Newton et al., 1986). Recently published results of much more extensive research in that laboratory have provided convincing evidence that memory is significantly and progressively impaired as hypocholinergic conditions persist (Russell et al., 1990). The experiments were designed to measure nine parameters of memory, ranging from what has been termed a primitive form of learning and memory, i.e., habituation (Heise, 1984), to the *de novo* acquisition of a conditional avoidance response (CAR) dependent upon differential reinforcement. Interestingly, the NADe diet had differential effects on habituation of locomotor activity and rearing behavior, having no effect on the former but blocking the latter. This finding suggests that different parameters of memory are not mediated by the same neurochemical processes.

Inhibitory (passive) avoidance has been used more widely than other behavioral assays in studying the effects of drugs and other treatments on learning and memory (*see* Section 4.2.). In the present experiments, the fact that replacement of ACh by the false transmitter occurred progressively throughout the experimental period means that it is not possible to differentiate among effects on learning, memory, or retrieval from memory. However, all of these involve processes in which memory is the common element. The present findings have three features of special interest. First, statistically significant differences between the NADe and control animals occurred only after 60 and 120 d on the diet, although the former had consistently poorer measures of memory throughout the experimental period. Second, the control group showed a consistent trend toward impaired performance during the testing period, a trend that appeared to be related to increasing age. Third, animals on the NADe diet also decreased systematically in their performance, but at a much faster rate than those on the control diet, reaching an asymptote by the 60-d assay.

Parameters of CAR performance provide evidence of impaired memory during the process of acquiring the avoidance response. The latter involved what is often referred to as reference memory. The animal had to master the general rule that the conditioned stimulus (CS) was a signal to move to the goal end of the straightaway; it was not required to remember what response it had made on earlier trials (working memory). In all three measures of the CAR, animals on the NADe diet were inferior in performance to the control subjects, the trends being apparent at all times, and statistically significant at 60 d and thereafter. NADe animals took more trials to learn the CAR, made more errors, and were slower in response times.

A report of the US National Institutes of Health Consensus Development Conference on Differential Diagnosis of Dementing Diseases (NIH, 1987) contains the statement that "...dementia is primarily a behavioral diagnosis." It is characterized by a decline in "...memory, other cognitive capacities, and adaptive behavior. In some demented patients, changes in motor, sensory, and visual systems appear. The most frequent of the dementing diseases are progressive in nature. In the experiments summarized briefly earlier, cholinergic hypofunctioning was induced progressively, and behavioral effects measured as observed in sensory, motor, and cognitive function. The results have shown that, as cholinergic hypofunctioning developed, complex behavioral functions were affected progressively, cognitive functions being most sensitive to change and showing the least adaptability.

When the animals on the NADe diets were given control diet, the effects on memory processes differed. Simpler processes returned to normal, whereas the more complex did not recover even after extensive normal feeding. These differential effects suggest that neural degeneration in key areas underlying the more complex behavioral tasks may have occurred. Experiments are under way to determine histological effects in animals maintained chronically on the NADe diet. Attempts to overcome the memory disturbance seen in the animals raised on the NADe diet by injection of drugs proposed to be beneficial in Alzheimer's disease are also under way.

Thus, it is clear that feeding rats a diet of the false ACh precursor, NADe, can result in significant memory deficits. This supports earlier work suggesting that memory in mice can be modulated by either increasing or decreasing the amount of choline in the diet (Bartus et al., 1980). However, the present approach is somewhat more naturalistic. There remain several unanswered questions; e.g., does the dietary treatment result in anatomical damage, as has been found for thiamine deficiency? However, work with this model is continuing and answers are not far away.

3.5. Acute and Chronic Drug Administration

It is well known that a wide range of drugs, including such psychotropic agents as ethanol and diazepam, can influence memory processes. However, there is not a great deal of interest in modeling these problems in animals, so no further discussion will take place on these agents. Similarly, although there is a considerable amount of literature on the use of scopolamine, a cholinergic antagonist, as a model of Alzheimer's disease, this research has been admirably reviewed by Smith (1988) and will not be dealt with further in this review. In this section, we will review the evidence in animals relating to potential mechanisms underlying the memory-disruptive effects of chronic treatment with either ethanol or the organophosphate pesticides.

3.5.1. Ethanol

Although thiamine deficiency, and not ethanol intake *per se,* has been recognized as the primary etiologic agent in Korsakoff's disease (Victor et al., 1971), it has been recognized that alcoholics without symptoms of Korsakoff's disease can also suffer from memory disorders (e.g., Butters, 1985; King, 1986; Victor and Adams, 1985). Although it has been difficult to dissect out the primary effect of ethanol from other factors associated with alcoholism in humans, some investigators have begun to look at neurochemical changes that might correlate with the neuropsychological changes seen in alcoholics. Freund and Ballinger (1989a,b), for example, have detected the loss of mAChR and benzodiazepine binding sites in several brain regions in alcohol abusers, replicating some earlier work, but not all (cf Nordberg et al., 1983; Perry et al., 1986; Tollefson and Gade, 1983). Arendt et al. (1983) reported extensive cholinergic cell loss in the NBM of patients with Korsakoff's disease, but not in chronic alcoholics without dementia. Thus, there is increasing evidence implicating cholinergic mechanisms in the neuropsychological deficits seen in chronic alcoholics. Recall that cholinergic mechanisms have also been suggested for Korsakoff's disease, but noradrenergic and serotonergic mechanisms have been suggested (Mair et al., 1988a; Overstreet and Russell, 1984).

Although there have been a tremendous number of studies in which chronic ethanol has been given to animals, relatively few have been specifically designed to provide information about the mechanisms underlying alcoholic dementia. Arendt et al. (1988a) recently exposed rats to 20% (v/v) ethanol (as sole drinking source) for 12 wk and then examined the brains. They noted losses of cholinergic-rich cells in the NBM, and reductions in CAT and AChE in the basal forebrain. These reductions were comparable to those found in rats with excitotoxic lesions of the NBM; thus the model may be useful for exploring the cholinergic hypothesis of alcoholic dementia. In a related study, Arendt et al. (1988b) demonstrated that the mode of ethanol administration used in their neuropathological studies also impaired performance on the radial arm maze. The cholinergic nature of this problem was reinforced by the finding that transplants of fetal cholinergic cells into the brains of the ethanol-treated rats could overcome the radial arm performance deficits (Arendt et al., 1988b; *see* Section 5.4.).

3.5.2. Organophosphates

With the virtual banning of the environmentally persistent organochlorine pesticides, there has been an upsurge in the use of organophosphate pesticides. The likelihood of exposure to such pesticides has therefore increased. Despite this, it has been difficult to relate specific behavioral and/or physiological effects in humans to organophosphate exposure, because individuals at risk are also often exposed to other agricultural chemicals, as well as automobile fuel and alcohol. Levin and Rodnitzky (1976), in an earlier review of the literature, pointed out that, if exposure was great enough to lower blood AChE activity, several changes in behavior could be detected, including vigilance, information processing, memory, speech performance, depression, anxiety, and irritability. Karczmar (1984) has indicated that these behavioral abnormalities seen with clinical exposure to organophosphates may persist for several months.

Because of the inability to relate specific clinical symptoms to organophosphate exposure in humans, animal studies can be useful. In fact, there are a considerable number of studies of the acute and chronic effects of organophosphates in animals, stimulated in part by contract money from the armed forces to research this topic (*see* Russell and Overstreet, 1987). However, the large majority of these papers have only slight relevance to the topic of an animal model of the organophosphate-exposed human. Consequently, we will focus on a limited aspect of this very broad research area. The reader is invited to consult the review by Russell and Overstreet (1987) for other aspects.

A key symptom mentioned above in organophosphate-exposed humans is memory disturbance. There is a reasonable amount of parallel evidence in animals as well. For example, Gardner et al. (1984) showed that rats chronically treated with diisopropyl fluorophosphate (DFP), an organophosphate, had impaired retention in a passive avoidance task. Loullis et al. (1983)

reported that the memory changes were related to the type of drug given chronically: if scopolamine, an antagonist, was infused chronically, rats exhibited enhanced memories during drug withdrawal; on the other hand, if physostigmine, an anticholinesterase, was infused chronically, rats exhibited impaired memories. Upchurch and Wehner (1987) have also reported memory deficits in mice chronically treated with DFP. It should also be pointed out that a go–no go operant task, which provides an index of working memory, was one of the few tasks for which complete tolerance to DFP was not observed (Overstreet et al., 1974; Russell and Overstreet, 1987).

The mechanisms underlying these memory dysfunctions have not been clearly established, but a strong possibility is the reduction in mAChR, which has been well documented to occur during chronic organophosphate exposure (*see* Russell and Overstreet, 1987). Such a mechanism would be consistent with the various reports indicating that organophosphate-treated rats are supersensitive to the behavioral, including memory-disruptive, effects of the cholinergic antagonists, scopolamine and atropine (Chippendale et al., 1972; Fernando et al., 1986; Raffaele et al., 1987,1990; Russell et al., 1971). It is particularly interesting that such supersensitivity to antagonists can be observed in animals treated with doses of organophosphates, which have no acute effects themselves (e.g., Bushnell et al., 1991; Chippendale et al., 1972; Raffaele et al., 1990).

Because there are memory disturbances in animals that have been chronically treated with organophosphates, it would appear that the decrease in mAChR concentrations, which have been widely recognized as likely mechanisms underlying the development of tolerance to organophosphates (*see* Russell and Overstreet, 1987), also has maladaptive consequences. No one has yet determined the duration of these effects, but Raffaele et al. (1990) have shown that they last for at least 15 d after the cessation of treatment. However, no one has attempted to counteract the memory disturbance by utilizing one of the treatments that have been used in other animal models of memory disturbance (*see* Section 5.). In fact, the prospect of using muscarinic agonists would not seem high because of the widely characterized subsensitivity to these agents in organophosphate-treated animals (Overstreet, 1984; Russell and Overstreet, 1987).

In sum, it is quite clear that both mAChR decreases and memory disturbance can be produced in animals chronically treated with organophosphates. Such animals are supersensitive to cholinergic antagonists, just as are Alzheimer's patients, who suffer from a different type of cholinergic abnormality (Sunderland et al., 1988). These parallel findings suggest that challenging individuals suspected of organophosphate exposure with scopolamine may help to reveal such exposure. The advantage of this procedure over the conventional blood AChE assay would need to be carefully considered. There are still some loose ends that the animal studies can help resolve; primary among them would be attempts to ameliorate the memory-disruptive effects of organophosphate exposure.

4. Techniques for Measuring Memory

As indicated in Section 2., learning and memory are behavioral processes that are interdependent with a number of other behavioral processes, including attention and arousal. Therefore, any consideration of the techniques for measuring learning and memory must also give some attention to techniques for measuring these associated processes. There is a wide variety of techniques available to measure both learning and memory and their associated behavioral processes, so it is impossible to cover them comprehensively in this section. Instead, the focus will be on those most frequently used in the animal studies.

4.1. Activity, Arousal, and "Memory"

The cholinergic system, which has been commonly implicated in learning and memory processes, has a longer history of involvement in arousal and behavioral inhibition (e.g., Carlton, 1963; Russell, 1966). In general, drugs that block mAChRs, such as scopolamine, produce disinhibition of behavior, including increases in activity. On the other hand, cholinergic agonists produce behavioral depression; this effect is one of the major handicaps to the use of such agents in the treatment of memory disorders (*see* Section 5.1.). Consideration of this literature suggests that any manipulation of the cholinergic system is likely to modify noncognitive behavioral processes as well as cognitive processes.

4.1.1. Activity Monitors

The measurement of activity in rodents has been used to monitor a wide variety of manipulations, and there is a plethora of devices, from observational open fields to very sophisticated electronic monitoring. Although the latter are certainly much more expensive than the former, they also reduce the need for technical support. There have been quite a number of reviews about the monitoring of activity in animals (e.g., Geyer et al., 1986; Reiter and McPhail, 1979; Robbins, 1977); their general conclusion was that the different devices may have measured different forms of activity, but that each device had a high degree of reliability. Each investigator will have to make his/her own decision about the type of apparatus to employ, depending on the technical and financial resources at his/her disposal.

4.1.2. Reactivity Monitors

The activity monitors described above measure the animal's basal activity with minimal external influences. It is also important to obtain some measure of the animal's reactivity to specific stimuli as well. Reactivity is commonly measured in rats by using a startle apparatus. The rats are placed in a chamber sitting on top of a pressure-sensitive strain gage. The movement of the rat to specific auditory or tactile stimuli is recorded (e.g., Overstreet, 1977). Alternatively, the animal's threshold to shock can be determined using a standard shock box (e.g., Crocker and Russell, 1984). It is important to use such devices to measure reactivity, because there is strong evidence that manipulation of the cholinergic system by systemically administered drugs can modify reactivity: agonists depress reactivity, and antagonists potentiate it (Crocker and Russell, 1984; Overstreet, 1977). However, investigators interested in developing animal models of memory disorders selectively damage forebrain cholinergic systems, and it is important to know whether these more selective treatments modify reactivity as well as learning and memory.

Other tests of sensory–motor coordination, such as swimming in a water maze, latency to enter a dark compartment on the training trial of a passive avoidance task, and sensitivity to shock, have generally revealed normal functions in lesioned animals (*see* Olton and Wenk, 1987). Thus, carrying out such tests is probably less critical than obtaining some measure of the animal's activity and reactivity. They could still serve as important negative findings in a study reporting changes in learning and memory.

4.2. Avoidance Tasks

There are two primary types of avoidance tasks: active avoidance requires the animal to make an active motor response to avoid shock, whereas passive or inhibitory avoidance requires the animal to be "passive" or inhibit responses to avoid shock. Since a common denominator for both tasks is shock, it is a good idea to test the animal's sensitivity to shock by independent means. Any manipulation that alters an animal's sensitivity to shock will most likely alter its performance in the avoidance task. Another procedure that can be used to determine whether a manipulation is truly altering memory is to conduct both active and passive avoidance tasks. A range of conditions are known to affect the two tasks in opposite directions; one example is the facilitation of active and disruption of passive avoidance produced by the cholinergic antagonists (e.g., Overstreet et al., 1988; Overstreet, 1989).

4.2.1. Active Avoidance

The most common form of active avoidance test is two-way active avoidance in a shuttle box. The animal must move back and forth between two identical compartments. A distinct tone or light is presented at the beginning of a trial, and a footshock is delivered after a few seconds if the animal has not moved. If the animal moves during the tone or light, an avoidance response is recorded; if it does not move until the shock comes on, an escape response is recorded. After the first trial is completed an intertrial interval of 20–30 s ensues, and on the next trial, the animal must return to the previously shocked compartment to avoid or escape the next shock. The conflict involved in avoiding shock by returning to an area where shock has been received previously has led some to suggest that this test, particularly when a tone is used as the warning stimulus, may be a useful test for studying anxiety (Criswell and Breese, 1989).

The reader will recall that cholinergic antagonists lead to facilitated acquisition in this task. Thus, it is not surprising that lesions of the cholinergic-rich areas of the NBM or medial septal area also lead to facilitated acquisition of this task (Hepler et al., 1985a,b; Kessler et al., 1986). Similar results have also been found after lesions of the septum or hippocampus (Gray and McNaughton, 1983). These findings are consistent with the notion mentioned above of a cholinergic inhibitory system (Carlton, 1963; Russell, 1966). However, Bailey et al. (1986) and McLamb et al. (1988b) reported that active avoidance acquisition was impaired after intrahippocampal injection of AF64A and colchicine, respectively. Therefore, there is a need for more studies using this task after manipulations designed to alter memory processes in animals. Most of the results to date can be accounted for by the model of a cholinergic inhibitory system; there is no need to invoke the concepts of learning and memory.

4.2.2. Passive (Inhibitory) Avoidance

In this test, the animal must inhibit a response that normally has a high probability of occurrence in order to avoid shock. A typical apparatus consists of a two-compartment box or runway. The animal is placed in the smaller, well-lit section, and on entering the darker section, it receives a footshock (03–1.0 mA for 0.2–2 s). It may be allowed to escape back into the safe, lighted section, or more commonly, it is removed and tested for retention some time later. Some investigators use brief (2-min) intervals in order to look at acquisition; i.e., how many trials does it require for the animals to reach a criterion of 3 min of remaining in the lighted section? Regardless of the training procedures, it is common to conduct a retention test at least 24 h after the acquisition trial(s); the rat is returned to the lighted section and is allowed up to 3–5 min to move into the dark section. A rat that has a high score has a good memory.

Because of the relative simplicity of this task and the short time required to test each animal, it has been widely used as an index of memory disruption. Lesions of the NBM and medial septal area by various approaches, treatment with AF64A to lesion the septohippocampal pathways, and injection of cholinergic antagonists are just a few of the manipulations that have led to a disruption of retention (memory) on the passive avoidance task (Bailey et al., 1986; Olton and Wenk, 1987; Overstreet and Russell, 1984; Smith, 1988). However, all of these treatments interfere with the functioning of cholinergic neurons, and the data may be accounted for by reference to the model of the cholinergic inhibitory system described above. The data on time between training and retesting lend support to the memory model; it has been reported that the deficits were minimal when the trials were closely spaced, were slight when the retention interval was 5 min, and were quite substantial at longer intervals (Olton and Wenk, 1987). The cholinergic inhibitory model cannot account for these time-dependent behavioral changes, whereas a memory model incorporating the consolidation concept can.

The passive avoidance test has proved to be a particularly useful test, but it should be used only in conjunction with other tests. It can be especially useful as an initial screening test. Most manipulations that have affected other, more sophisticated tests have also disrupted passive avoidance retention.

4.3. Operant Tasks

Operant tasks are performed in special chambers that contain food cups or water dippers, and one or more levers for the animal to press under various conditions. Because they involve food or water reward, they require deprivation schedules. Rats are commonly reduced gradually to 85% of free feeding weight before being trained in the task. Investigators utilizing these tasks should also study the effect of any treatments on the animals consummatory behavior, since any manipulation that influences food and/or water motivation is likely to affect performance on this task.

4.3.1. Delayed-Match-to-Sample

This task is carried out in a standard operant chamber with two retractable levers. After initial training to respond on both levers to obtain food, the animal is started on the task. A sample lever is available until the rat presses it. Then it is retracted, and shortly thereafter both levers become available; a press of the sample lever will deliver a food pellet. The investigator can vary the duration of the delay between the first lever press and the second opportunity (Bushnell, 1988). In fact, such a manipulation is necessary to obtain information about the manipulation on memory processes. This test has not been widely used in this field, possibly because it requires both sophisticated equipment and dedicated technical support, but it has provided some useful information about the effects of treatments on memory function. For example, it has been reported that lesions of the NBM impair performance on this task, particularly at longer delay intervals (Dunnett, 1985). Bushnell et al. (1991) has found that this task was particularly useful in studying rats chronically treated with organophosphates; no effects were seen initially, but memory impairment developed at about the time a significant decrease in mAChR would have been expected (6–10 d).

4.3.2. Temporal Discrimination

Meck et al. (1987) reported that ibotenic lesions of the NBM and medial septal area, two different cholinergic projection regions, produced differential effects on temporal memory. The task involved training the rat to perform a standard fixed-interval operant task, in which the first lever press after a specific interval was rewarded with food. Then the rats were given extinction-like trials, during which a press did not result in food delivery, in order to assess response functions. Rats with NBM lesions responded later, whereas those with lesions in the medial septal area responded sooner than controls, suggesting opposite types of reference memory deficits. Tests for working memory found that only the lesions in the medial septal area produced a deficit (Meck et al., 1987). This test also required both sophisticated equipment and dedicated personnel, so it has not been used very often—even though it has provided very useful information about the two cholinergic projection pathways.

4.3.3. Wisconsin General Test Apparatus

The Wisconsin General Test Apparatus is not strictly an operant task, but has some functional similarity to such tasks, particularly regarding the delayed-match-to-sample paradigm. This apparatus is commonly used in testing primates, and has been employed by Ridley and colleagues in studies of cholinergic involvement in learning and memory in marmosets (e.g., Ridley, 1984a,b,1985,1987). This apparatus has two food wells that can be covered by visually discriminable objects. A screen is available to permit the changing of the conditions on each trial without being seen by the animal. The test can be conducted as a standard discrimination test, with one object of a pair always covering the food well with the reward. Alternatively, a delay-match-to-sample paradigm can be conducted, in which the food is always in the well it was in on the first trial. Ridley et al. (1984a,b,1985,1987,1988a,b) have lesioned both cholinergic pathways in the marmoset and have reported differences on behaviors measured by the Wisconsin General Test Apparatus. For example, lesion of the diagonal band of Broca, which projects to the hippocampus, does not impair learning of a repeated trial object discrimination (Ridley et al., 1988b), whereas lesions of the NBM do (Ridley et al., 1984a,b,1985,1987).

4.4. Maze Tasks

The maze has long been used to test the behavior of rodents. With greater sophistication in design and approach, mazes continue to be useful in studying the consequences of various manipulations on learning and memory processes. Mazes can be of many different designs, but most require some form of motivation to elicit performance from the rat. By far, the largest majority of investigators deprive their rats of food and use some especially desirable food as a reward (e.g., fruit loops). A number of investigators have used escape from water as the motivation, and Kant et al. (1988) have argued for the superiority of such tasks over food-rewarded paradigms. In any case, it is important for each investigator to demonstrate that any manipulation employed to modify memory process has little or no effect on the conditions used to motivate the animal.

4.4.1. Spontaneous Alternation in a T Maze

This task employs a T maze with a runway and two identical choice arms. As the term implies, the rat is not deprived, and no reinforcement is given. The rat may be permitted to choose one arm on the first trial, or it may be forced into one arm. On the second trial, the rat is given a choice and selects the alternate arm on about 80% of the trials. Spontaneous alternation is reduced by treatments that impair the function of the cholinergic system (e.g., Olton and Wenk, 1987; Overstreet et al., 1988).

4.4.2. Delayed Alternation in T Maze

This task is a variation of the spontaneous alternation task; the rat is deprived of food and is given a choice on all trials, except that it is rewarded for going to the alternate arm to the one visited on the previous trial. The test can be conducted as a continuous task, in which each trial is dependent upon the location of the food on the previous trial, or as a discrete-trial paradigm, in which pairs of trials are given, the performance on the second trial being dependent on the location of the food on the first trial. Animals with lesions in the NBM or medial septal area are often impaired on these tasks, but the degree of impairment may vary with the time interval between trials and the duration of the test (Olton and Wenk, 1987).

4.4.3. Spatial Discrimination in a T Maze

In this task, the same arm always contains the food, and the rat must learn to discriminate that arm from the unrewarded arm. There is very little evidence for an impairment on this type of task following disruption of cholinergic function in rats (Olton and Wenk, 1987), but there is in marmosets using the Wisconsin General Test Apparatus (e.g., Ridley et al., 1984a). Thus, there is a need for additional studies in this area.

4.4.4. Radial Arm Maze

This apparatus, a maze with a central platform and various arms (8 or 16 usually) radiating outward, has been very frequently used in studies of animal models of memory disturbance. A number of reviews emphasizing special applications have recently been published (Levin, 1988; Walsh and Chrobak, 1987). This test is commonly conducted with food reward, so it is important to determine any effect of the manipulation on food motivation as well as upon radial arm maze performance. The comments below are directed to the eight-arm maze.

A typical procedure in the radial arm maze is to bait all eight arms and record the animal's efficiency of performance; each arm revisited before obtaining seven of the eight food pellets is counted as an error. Typically, rats have several errors on the first day, but continue to improve on subsequent days. Sometimes, the rats are taken out after consuming four of the pellets and returned after a variable delay. Rats with NBM lesions exhibit greater impairments on this task when the delays are long (Olton and Wenk, 1987).

Another procedure that can be used in the radial arm maze task is to bait only a limited number of the arms (three or four) and measure the animal's performance. Over time (days), the animal learns which arms are baited and which are not—a type of reference memory. In addition, the animal must remember which arms it has visited on any particular day; any return to a previously visited arm is a working memory error. Thus, it is possible to obtain information about both types of memory.

4.4.5. Water Mazes

The most common form of water maze consists of a circular tank of water with a hidden platform. The rat is placed in the water at different places and allowed to find the platform. The amount of time (latency) to reach the platform and the number of visits to the quadrants not containing the platform (errors) are recorded. Then the platform may be moved, and performance recorded again. Initial studies suggest that lesions of the NBM impair performance in the circular water maze, but more work is needed (Olton and Wenk, 1987). It is well known that cholinergic antagonists also impair performance on this task (e.g., Lindner and Schallert, 1988).

Another type of water maze is functionally equivalent to the food mazes described above. For example, the rat could be rewarded (reach the platform) by selecting the right arm, the one with a distinctive visual stimulus, or the opposite arm to the one selected on the previous trial. Delay intervals can be interposed, so that the effects of time can be examined. Water mazes have been less commonly used than food mazes, but, as indicated previously, there is some evidence that they may have some advantages (*see* Section 3.4.2.; Kant et al., 1988).

4.4.6. Other Tasks

A range of other tasks have been used in studies of animal models of memory disorders. Because their use has been limited, we will not discuss them here. The interested reader may wish to consult Olton and Wenk (1987) for a brief discussion of some of them.

5. Techniques for Potential Treatments

Alzheimer's disease has had a poor treatment history compared to Parkinson's disease, but has fared equally as poorly as other neurodegenerative disorders, such as Huntington's disease and amyotrophic lateral sclerosis (motor neuron disease). Because of this relatively poor treatment history, there is considerable interest in developing animal models that accurately mimic Alzheimer's disease in the hope that treatments developed to overcome the memory impairments in animals will be of use clinically. We will confine our attention to four approaches that have been used with increasing success in recent years. Recent reviews of other approaches are available (e.g., Crook et al., 1986; Olton and Wenk, 1988,1989), and the interested reader may wish to consult them.

5.1. Cholinergic Agents

By far, cholinergic agents have been the most commonly used technique in attempting to overcome the memory-disruptive effects of experimental treatments in animals and disease states in humans. Consequently, this literature has been reviewed often, most recently by one of us (Overstreet, 1989). Therefore, this section will be brief and will focus on animal studies that were not included in recent reviews because of their recency.

5.1.1. Precursors

In general, treatment of animals or humans with the acetylcholine precursors, choline and lecithin, has had little effect by itself. There is no evidence of any benefits from more recent studies either. So, it appears that the hope that Alzheimer's disease would resemble Parkinson's disease and be amenable to treatment with precursors has not been fulfilled (Bagne et al., 1986; Overstreet, 1989).

5.1.2. AChE Inhibitors

The response of animals and humans to the AChE inhibitors, e.g., physostigmine and tetrahydroaminoacridine (THA), has been much better than that following the precursors, but there is some dispute about whether any clinically significant benefits have arisen in humans (Bagne et al., 1986). Physostigmine was reported to be effective in only 3 out of 12 studies (Overstreet, 1989), and the large-scale clinical studies currently under way with THA have not revealed any useful information as yet. In animals in which the cholinergic system has been disrupted by lesions or drug treatments, physostigmine has been quite successful in overcoming the memory disruption (e.g., Murray and Fibiger, 1985,1986; Miyamoto et al., 1989; Ogura et al., 1987; Overstreet, 1989; Tilson et al., 1988a; Ueki and Miyoshi, 1989). The paper by Miyamoto et al. (1989) is noteworthy in that they chronically infused physostigmine and other agonists in the rats, as Harbaugh et al. (1984) have reported in humans. Such a mode of administration largely overcomes the depressive side effects that AChE inhibitors can have (e.g., Murray and Fibiger, 1985).

The fact that AChE inhibitors are quite effective in overcoming the memory deficits in lesioned rats, but are only partially effective at best in humans has been highlighted as a possible deficiency of the animal models (Smith, 1988). These differences could just as well reflect the difficulty in carrying out memory-enhancement studies in humans, so there should not be too great a pessimism about the animal models. What is remarkable is that there are large-scale clinical trials of the effects of THA in humans, yet there do not appear to be any studies of this drug in animal models of memory disorders. There is certainly a need for further studies in this field.

5.1.3. Cholinergic (Muscarinic) Agonists

The use of directly acting mAChR agonists in ameliorating the effects of lesions or other treatments on memory processes in animals has been comparatively recent, as has their use in the attempted treatment of Alzheimer's disease. These compounds suffer from pharmacological features that make their use difficult; these include relatively short durations of action and particularly steep dose–response curves. Potter and Ferrendelli (1989) recently called attention to another problem: virtually all of the tertiary (necessary for penetration to the brain) mAChR agonists previously used in treating Alzheimer's disease are only partial agonists at the phosphatidylinositol second messenger system. Given these inherent pharmacological problems, it is not surprising that little or no benefits have been reported for these compounds in humans (Bagne et al., 1986; Overstreet, 1989).

However, as was the case with physostigmine, these directly acting agonists have been quite effective in overcoming the memory disruption in lesioned animals. Improvement or amelioration has been observed with agonists, such as pilocarpine (Murray and Fibiger, 1986), oxotremorine (Miyamoto et al., 1989), RS-86 (Tilson et al., 1988a), and arecoline (Ridley et al., 1986,1987). Thus, there appears to be another dissociation between the human and animal studies, with the directly acting agonists being effective in animals, but not in humans. Once again, it is tempting to view this outcome as a handicap for the animal models. It is also possible to view the animal studies with guarded optimism; perhaps they will stimulate more concerted efforts to examine the effects of these agonists on Alzheimer's patients. The studies with directly acting agonists completed to date are really quite limited.

A final point that should be made in this section is the question of specificity. As remarked earlier, investigators are not always successful in limiting their destruction to only cholinergic neurons, so other neurotransmitter systems may participate (*see* Wenk et al., 1988). A similar argument applies to attempts to overcome the memory deficits; drugs interacting with noncholinergic neurotransmitter systems may be effective. As yet, little attention has been given to this idea (*see* next section). It is pertinent to know that only cholinergic drugs were able to ameliorate the effects of intra-NBM colchicine on radial arm maze performance; naloxone and vasopressin did not (Tilson et al., 1988a). This is certainly an area for further research; not only will the results impinge upon the cholinergic model of memory disturbance, but also they could lead to new, effective therapies for Alzheimer's disease.

5.2. Other Drugs

A wide range of other drugs have been used in elderly humans and/or aged animals in an attempt to overcome the cognitive deficits. Compounds include metabolic enhancers, such as piracetam; vasodilators, such as dihydroergotonine; enhancers of cerebral circulation, such as papaverine, and drugs relatively specific for particular neurotransmitter systems, such as the serotonin reuptake blocker, alaproclate, and the α_2-adrenoceptor agonist, clonidine (Altman and Normile, 1986; Bagne et al., 1986; Zornetzer, 1986). However, because of space limitations and, more importantly, because of the rare use of these compounds in the animal models discussed above, only a few brief comments will be made in this section. The reader is referred to the reviews mentioned above, as well as the more recent reviews by McGaugh (1989), Olton and Wenk (1988,1989), and Robbins and Everitt (1987) for further details.

Because the cholinergic neurons are quite obviously degenerating in Alzheimer's patients, many investigators have focused on cholinergic agents to overcome the memory deficits. However, Flood and Cherkin (1986) showed that a range of noncholinergic drugs could overcome the memory disturbance in mice produced by the cholinergic antagonist, scopolamine. Therefore, it is possible that drugs acting upon other neurochemical systems may be able to ameliorate the memory disturbance produced by lesions of cholinergic neurons. Sadly, there appear to be no studies of this possibility in the animal models discussed above, except for the report by Tilson et al. (1988b). This is certainly a fertile field for further research. Another area that has been largely neglected is the possibility of cotreatment with cholinergic and noncholinergic drugs (*see* Altman and Normile, 1986; Zornetzer, 1986). Now that there are a number of sound animal models of memory dysfunction, such studies are quite feasible.

5.3. Transplants

Although neuronal grafting began with an interest in replacing the degenerating dopamine neurons in Parkinson's disease (*see* Bjorklund et al., 1983), interest quickly turned to the cholinergic system and Alzheimer's disease. Since the early discovery that implants of septal grafts into the hippocampus of aged rats could ameliorate learning impairments (Gage et al., 1984), additional studies have confirmed the efficacy of grafts in aged rats (Dunnett, 1988,1989; Dunnett et al., 1988; Gage and Bjorklund, 1986). It is not the purpose here to provide a long discussion about the mechanisms underlying the effectiveness of the grafts; however, it is worthwhile pointing out that one suggestion is that the grafts contain nerve growth factors, which stimulate growth in the damaged neurons (*see* next section). The interested reader may wish to consult the more detailed discussion by Dunnett and Bjorklund (1987).

Most of the earlier transplant studies were conducted by Bjorklund or workers trained in his laboratory. More recently, additional investigators have become involved, and the results have remained impressive, so much so that a recent collection of articles has come out in monograph form (Gage et al., 1989). Among the findings are that the grafts can ameliorate the consequences of NBM lesions (Dunnett, 1988), amygdaloid lesions (Ermakova et al., 1989), AF64A treatment (Ikegami et al., 1989), or chronic alcohol treatment (Arendt et al., 1988b). Thus, there is reason for optimism regarding the animal studies. Whether such procedures can ever be used in humans is debatable; there are both technical and ethical issues involved. No doubt this debate will continue as more laboratories report positive results of neuronal grafting.

5.4. Nerve Growth Factors

Recently, a lot of interest has been generated by the recognition that cholinergic neurons, particularly those projecting from the septum to the hippocampus, are influenced by nerve growth factor (Hefti and Knusel, 1988; Hefti and Weiner, 1986). There has also been a lot of interest in the gangliosides as compounds that may also influence cholinergic and other neurons (Ando, 1983). These two groups of compounds may have complementary roles in normal functioning of the nervous system (Casamenti et al., 1989). Nevertheless, we will consider them in separate sections.

5.4.1. Nerve Growth Factor (NGF)

As indicated above, there is a tremendous amount of basic work taking place at present on NGF and related nerve growth factors, so a discussion of the interaction between NGF and cholinergic neurons will be giving a very limited picture of NGFs role. Nevertheless, such a discussion is necessary from our perspective of potential treatments for memory disorders in humans. Could NGF be considered a possible treatment of Alzheimer's disease? The answer is yes, quite definitely (e.g., Hefti and Weiner, 1986). However, much information is necessary from animal models. Although there is a good deal of information from neurochemical and neuroanatomical studies that NGF can influence cholinergic neurons (e.g., Batchelor et al., 1989; Dreyfus, 1989), there are comparatively few behavioral studies. Recently, Mandel et al. (1989) reported that spatial learning in rats, which was impaired by NBM lesions, was improved following NGF treatment. No doubt, this positive finding will be the first of many studies designed to examine the behavioral consequences of NGF administration in lesioned rats. Until there are additional positive findings, any conclusion other than that NGF appears promising would be premature.

5.4.2. Gangliosides

Gangliosides are composed of glycosphingolipids, which, among other functions, participate in the repair of the nervous system following injury (Ando, 1983). Monosialoganglioside

(GM1), one member of this class, has been reported to promote the regeneration of damaged nerves and alter the extent of behavioral, biochemical, and neuroanatomical effects following neuronal damage (e.g., Karpiak, 1983; Toffano et al., 1983). It is not surprising, therefore, that GM1 also promotes the recovery of cholinergic neurons following damage (e.g., Pedata et al., 1984).

Several behavioral studies have now been completed in animal models (lesioned or modified to produce memory disturbance) following administration of GM1 or related gangliosides. All studies to date indicate that there is substantial behavioral improvement in such animals, which is often paralleled by neurochemical changes, but not necessarily by neuroanatomical recovery (e.g., DiPatre et al., 1989; Emerich and Walsh, 1989; Walsh et al., 1989). Again, as with the NGF studies, the behavioral data are very sparse, and more work is necessary before detailed conclusions can be made. It is appropriate to suggest that the gangliosides in general and GM1 in particular are potentially useful therapeutic agents.

6. Summary

Table 1 provides a brief summary of animal models for six conditions in humans that are well documented to result in memory disorders. It can be seen that the animal models mimic the human conditions quite well in many respects, but there are continuing questions about the specificity of some of the approaches. Another problem is that the relative success of treating memory disorders in the animal models with cholinergic agonists has not been paralleled by a similar success in humans (*see* Smith, 1988). Whether this lack of correspondence will also apply to transplants and/or neural growth factors must await further research. In addition, there may be ethical and technical barriers to overcome before these promising treatments can be used on humans.

Table 1 also indicates that there have been very little data about the prospect of treating non-Alzheimer's-type memory disorders. Therefore, this appears to be a fertile field for further research. Also, as indicated in Section 5.2., very few researchers

Table 1
Animal Models of Memory Disorders

	Animal model		
Human condition	Approach	Correspondence	Treatment
Alzheimer's	Excitotoxins	Good; specificity?	ACh agonist
	AF64A	Fair; specificity?	ACh agonist
	Colchicine	Insufficient data	ACh agonist
	Dietary	Good; anatomy?	N.A.[a]
Korsakoff's	Lesions	Good	N.A.
	Dietary	Good	N.A.
Global amnesia	Lesions	Good	N.A.
ECT-induced	ECS	Good	N.A.
Alcoholic dementia	Ethanol	Fair/Good	Transplant
Organophosphate-induced dementia	Chronic OP	Fair/good	N.A.

[a] N.A. = Data not available.

have tested the effects of noncholinergic drugs in their animal models, even though they may have met with some success in treating humans. Much has been accomplished in the development of animal models of memory disorders; much more remains to be done.

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